# Air Quality Management Training Program

# Toxicological Evidence of Air Pollution Health Effects


## Jesús A. Araujo, MD, PhD

Associate Professor of Medicine (Cardiology) &
Environmental Health Sciences




* No conflicts of interest to disclose *

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# Outline

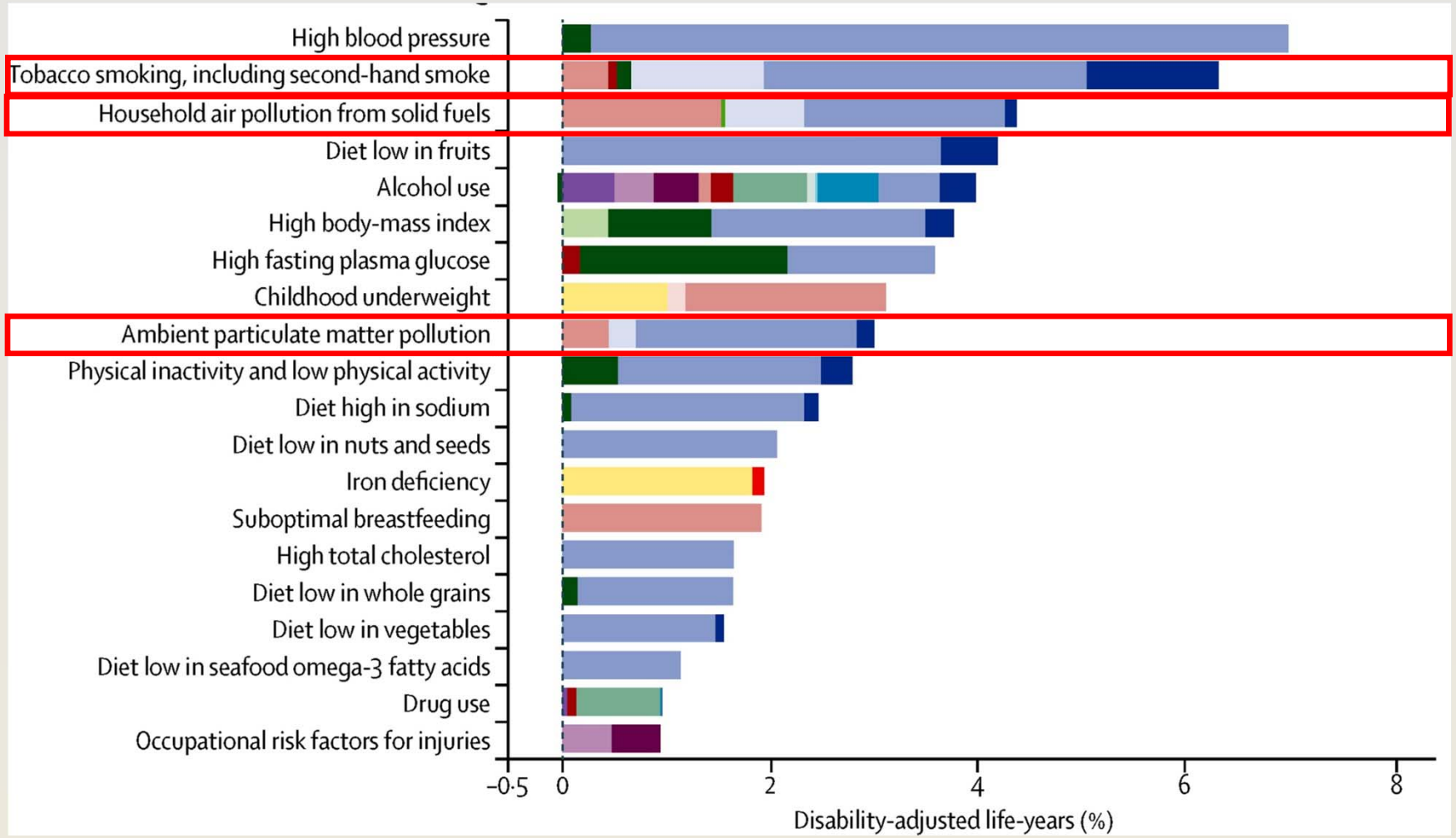
I) Air Pollution and Health

II) Toxicological Evidence

- a. Approaches to study health effects
- b. Effects in the lungs, vasculature, metabolism
- c. New investigational approaches

III) Summary and Perspectives

# 2010 Global Burden of Disease




DALY = YLL (Years of Life Lost) + YLD (Years Lived with Disability)

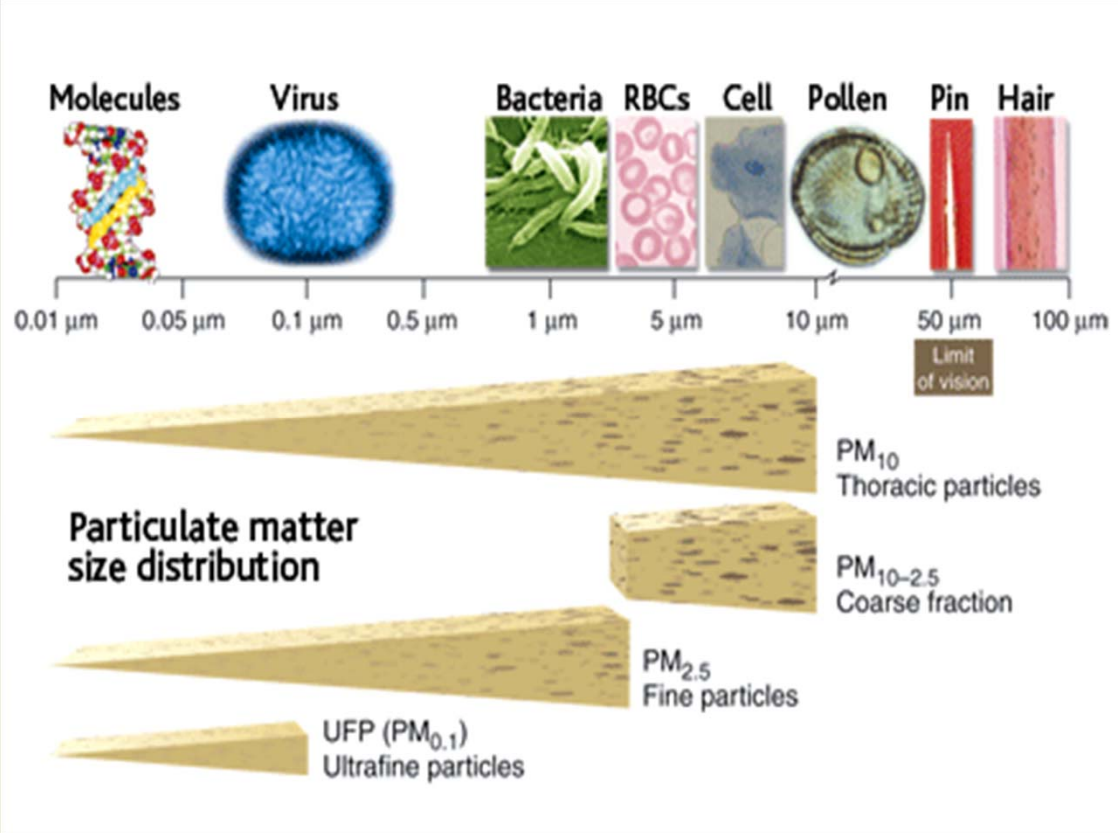
*Lim et al, Lancet 2012; 380: 2224-60*

# Ambient Air Pollutants



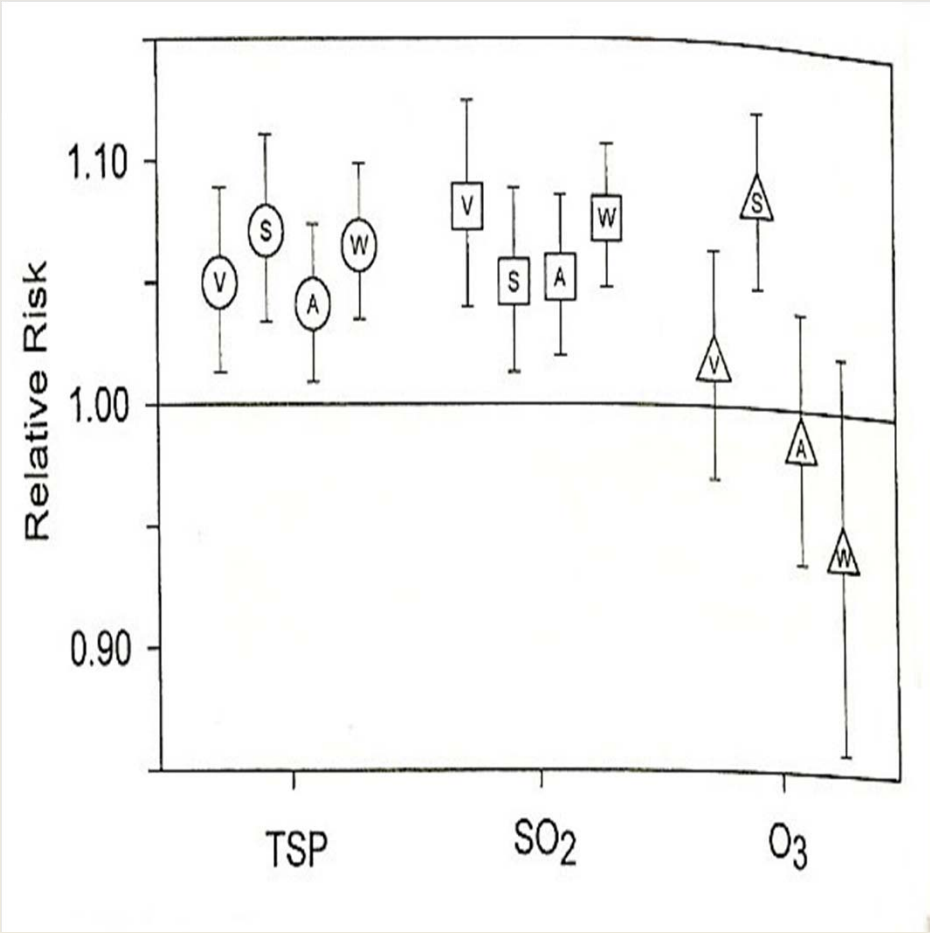

**Air Pollution**

- **Gaseous**
- **Particulate**
  - **TSP**
    - **$PM_{10}$ (< 10 μm)**
      - **Fine ($PM_{2.5}$)**
        - **UFP ($PM_{0.1}$)**
      - **Coarse ($PM_{10-2.5}$)**

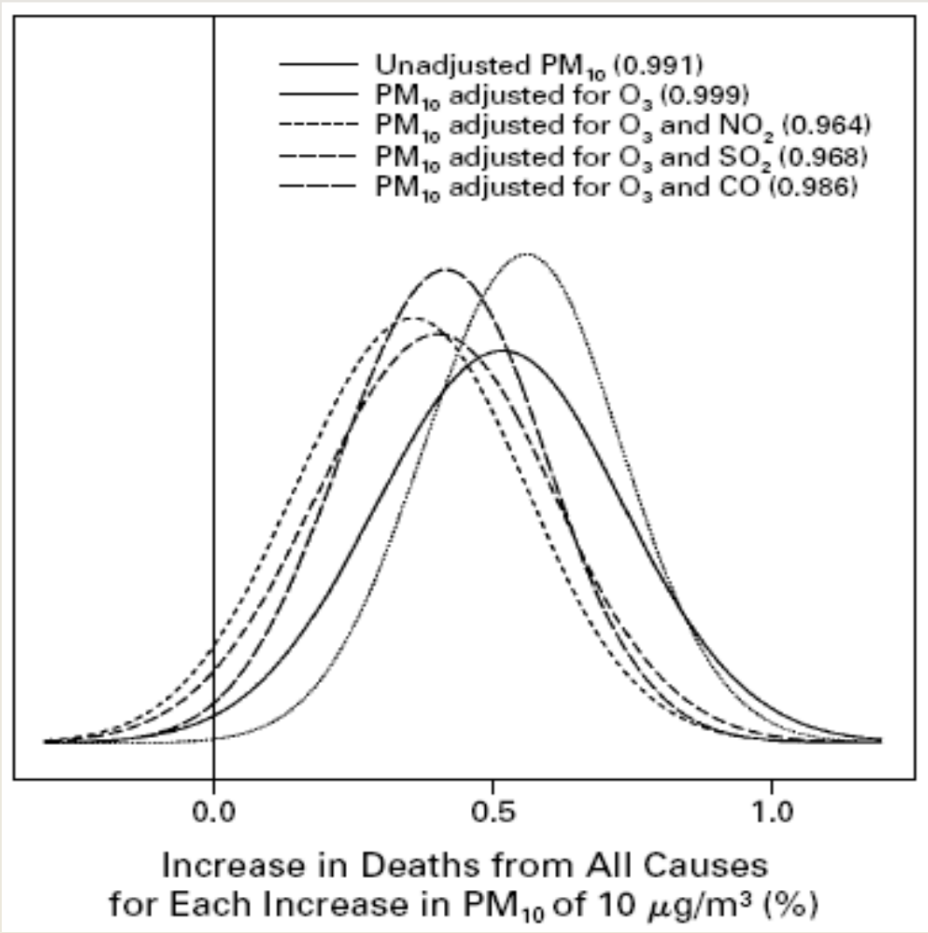



*Brook et al, Circulation 2004; 109: 2655*

# Air pollution and total mortality




*Dockery & Schwartz, Epidemiol 1995*




*Samet et al, NEJM 2000*

# $PM_{2.5}$ and cardiovascular M & M

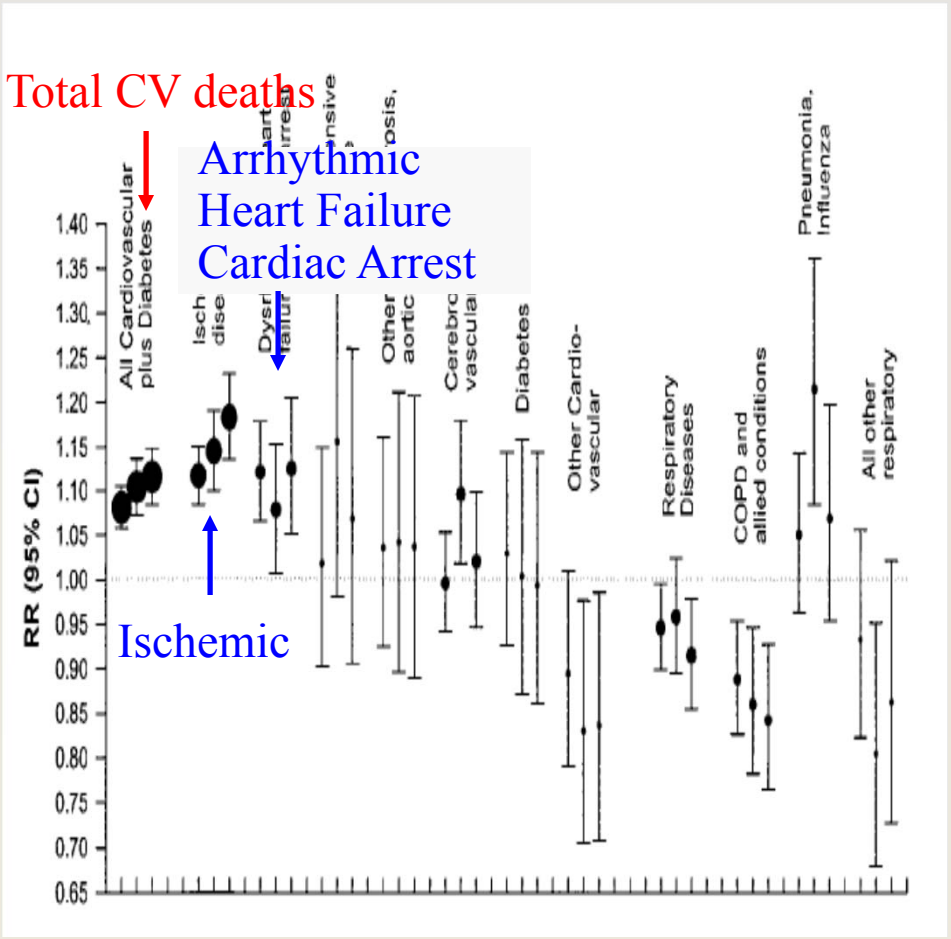


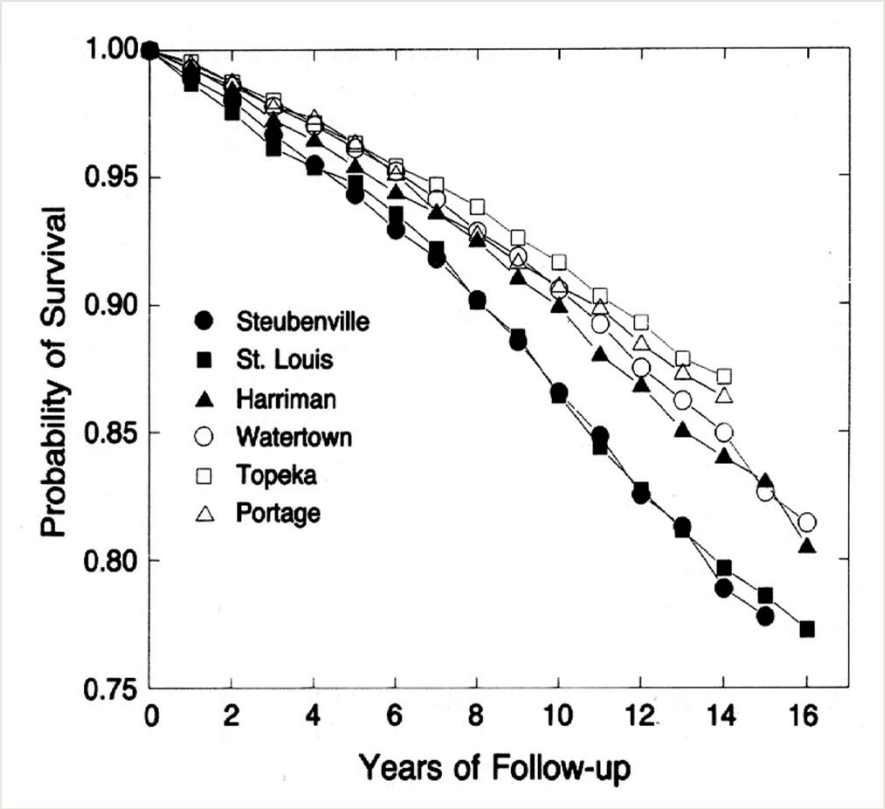

Table 3. Estimated Hazard Ratios for the Time to the First Cardiovascular Event or Death Associated with an Exposure Increase of 10 μg per Cubic Meter in the Level of Fine Particulate Matter ($PM_{2.5}$).*

| Outcome | No. of Events | Hazard Ratio (95% CI) | | |
|---|---|---|---|---|
| | | Overall | Between Cities | Within Cities |
| **First cardiovascular event** ↑24% | | | | |
| Any cardiovascular event† | 1816 | 1.24 (1.09–1.41) | 1.15 (0.99–1.32) | 1.64 (1.24–2.18) |
| Coronary heart disease‡ | 1268 | 1.21 (1.04–1.42) | 1.13 (0.95–1.35) | 1.56 (1.11–2.19) |
| Cerebrovascular disease§ | 600 | 1.35 (1.08–1.68) | 1.20 (0.94–1.54) | 2.08 (1.28–3.40) |
| Myocardial infarction | 584 | 1.06 (0.85–1.34) | 0.97 (0.75–1.25) | 1.52 (0.91–2.51) |
| Coronary revascularization | 949 | 1.20 (1.00–1.43) | 1.14 (0.93–1.39) | 1.45 (0.98–2.16) |
| Stroke | 554 | 1.28 (1.02–1.61) | 1.12 (0.87–1.45) | 2.08 (1.25–3.48) |
| **Death from cardiovascular cause** ↑76% | | | | |
| Any death from cardiovascular cause | 261 | 1.76 (1.25–2.47) | 1.63 (1.10–2.40) | 2.28 (1.10–4.75) |
| Coronary heart disease | | | | |
| Definite diagnosis | 80 | 2.21 (1.17–4.16) | 2.22 (1.06–4.62) | 2.17 (0.60–7.89) |
| Possible diagnosis | 59 | 1.26 (0.62–2.56) | 1.20 (0.54–2.63) | 1.57 (0.29–8.51) |
| Cerebrovascular disease | 122 | 1.83 (1.11–3.00) | 1.58 (0.90–2.78) | 2.93 (1.03–8.38) |

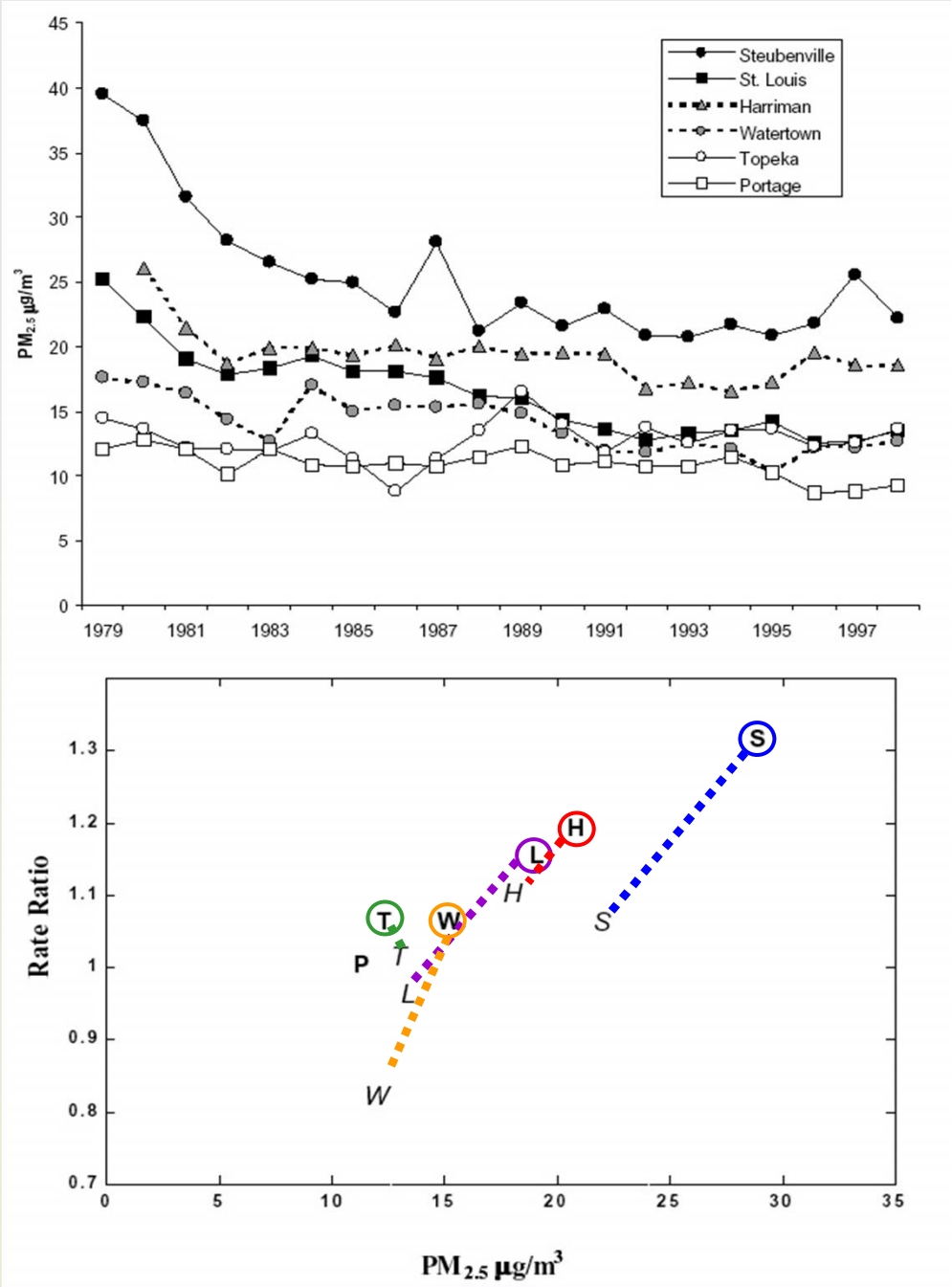
*Pope et al, Circulation 2004; 109: 71*

*Miller et al, NEJM 2007; 356: 447*

# Harvard six cities study

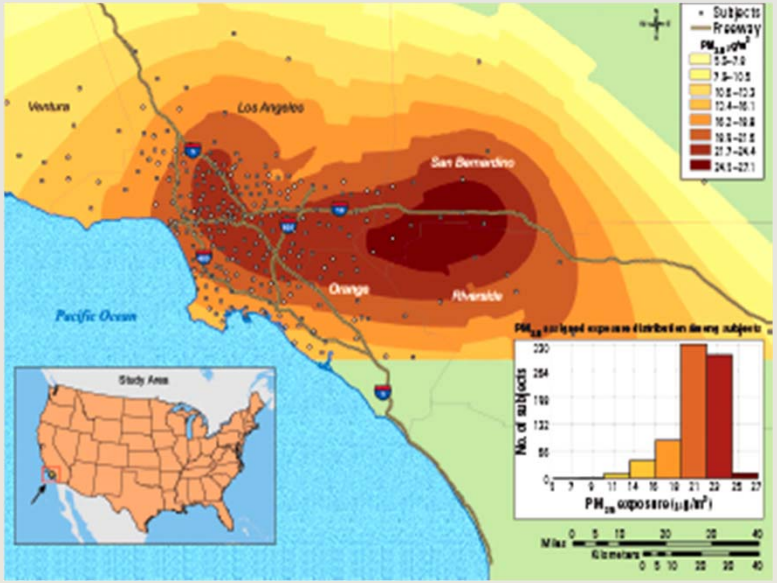


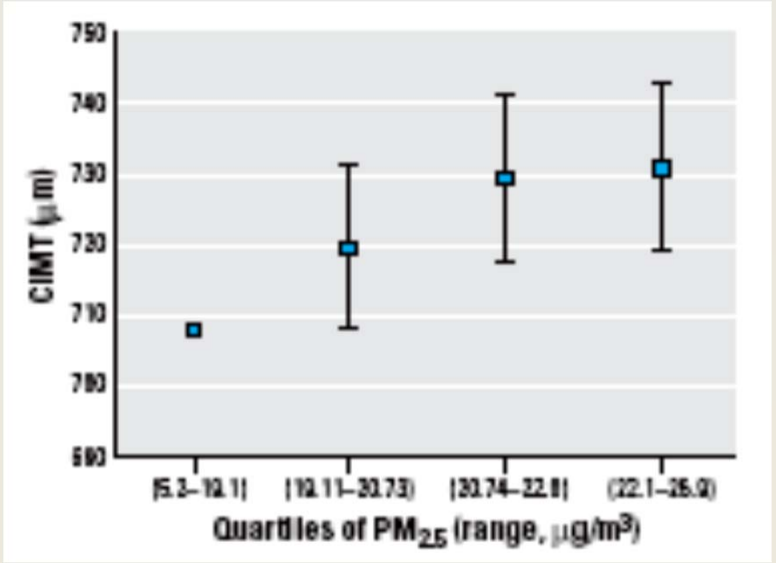

Dockery et al, NEJM 1993



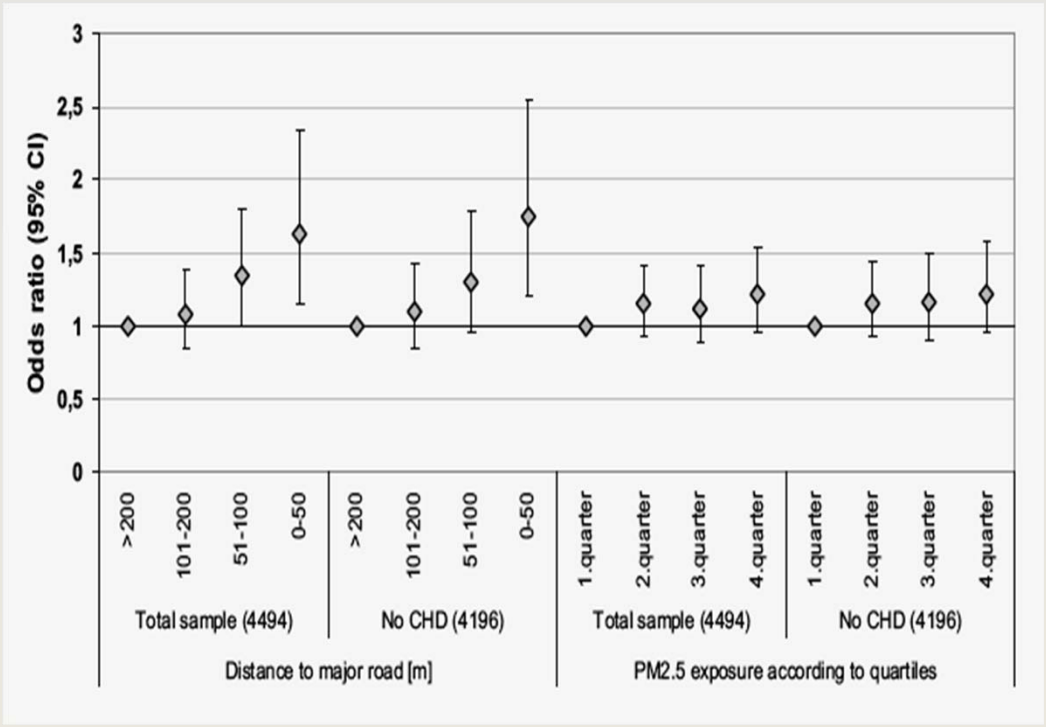

Laden et al, AJRCCM 2006

# $PM_{2.5}$ and atherosclerosis




Kunzli et al, EHP 2005

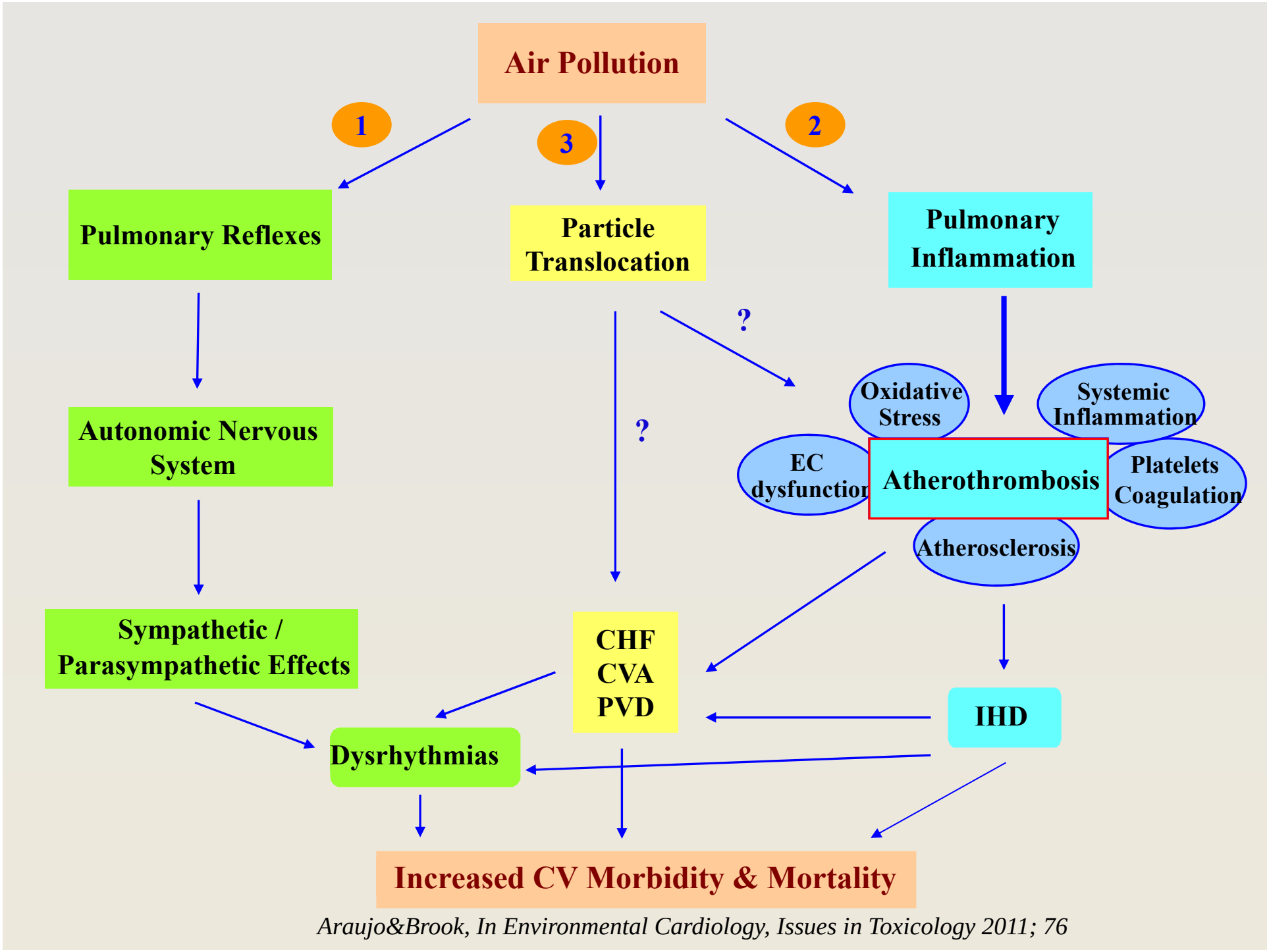



Hoffman et al, Circulation 2007

**Multi-Ethnic Study of Atherosclerosis**

3% increase in CIMT per each 12 mcg/$m^3$ increase in mean annual $PM_{2.5}$

Diez Roux et al, Am J Epidemiol 2008




*Araujo&Brook, In Environmental Cardiology, Issues in Toxicology 2011; 76*

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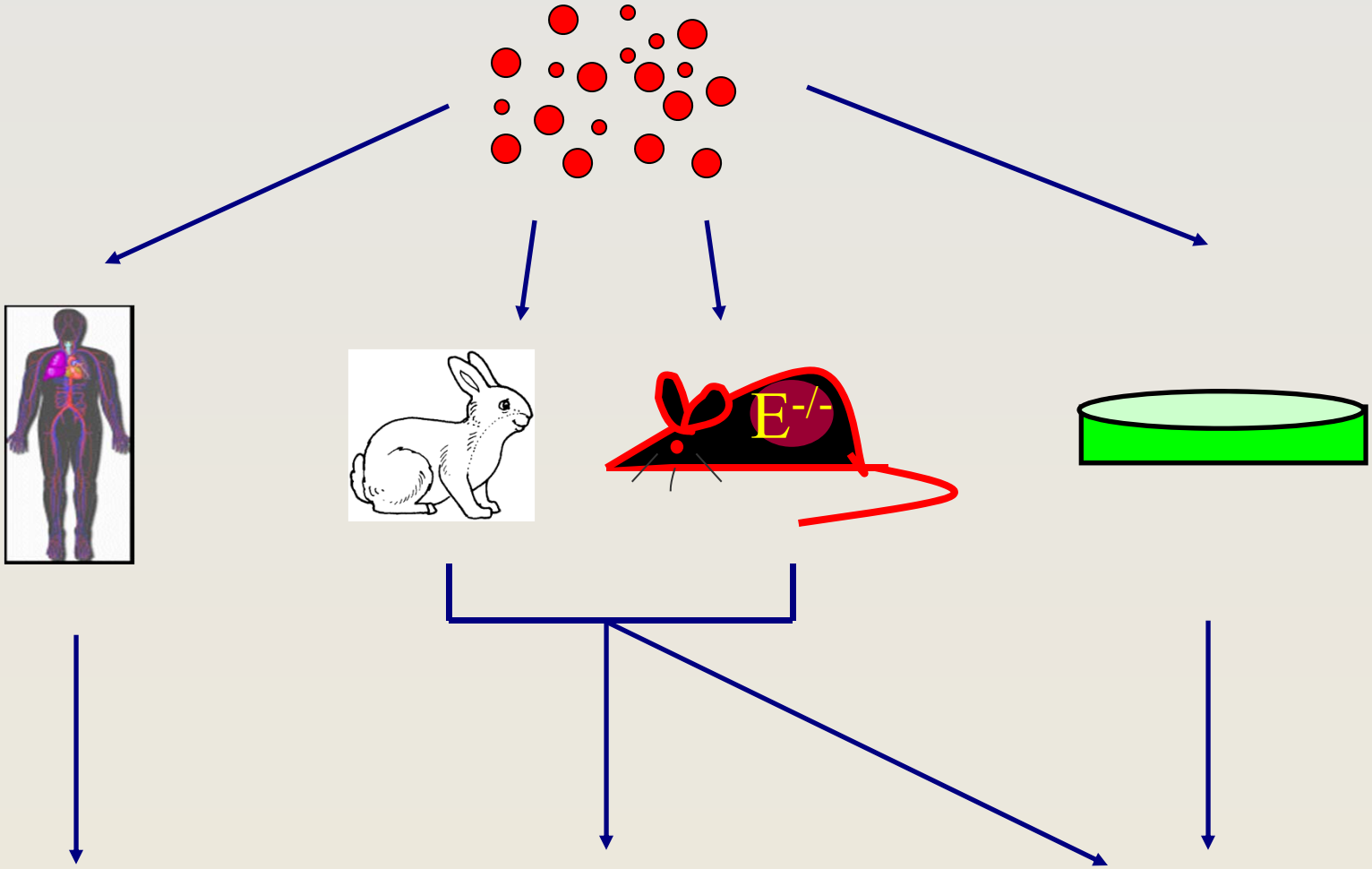
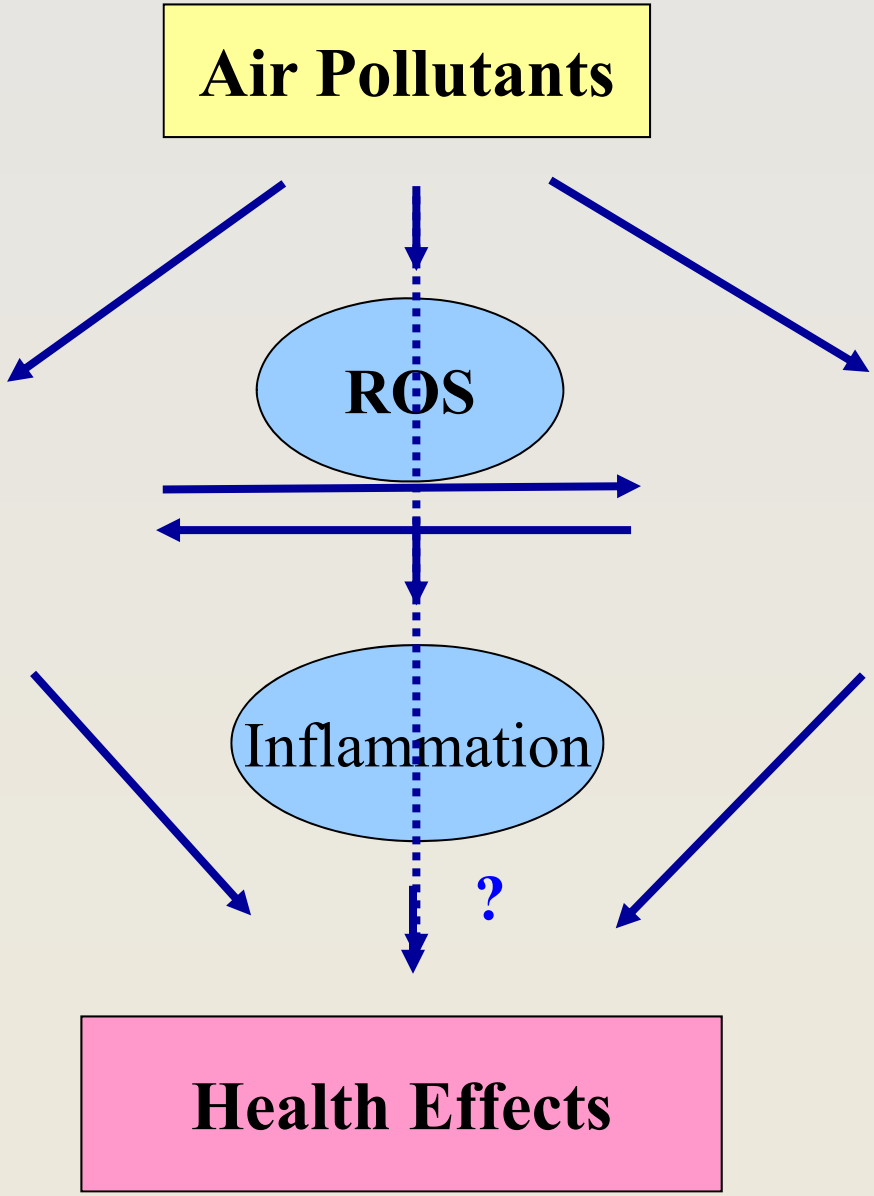
# How to Study Health Effects Induced by Air Pollutants?



# Mechanistic Models for the involvement of ROS and Inflammation



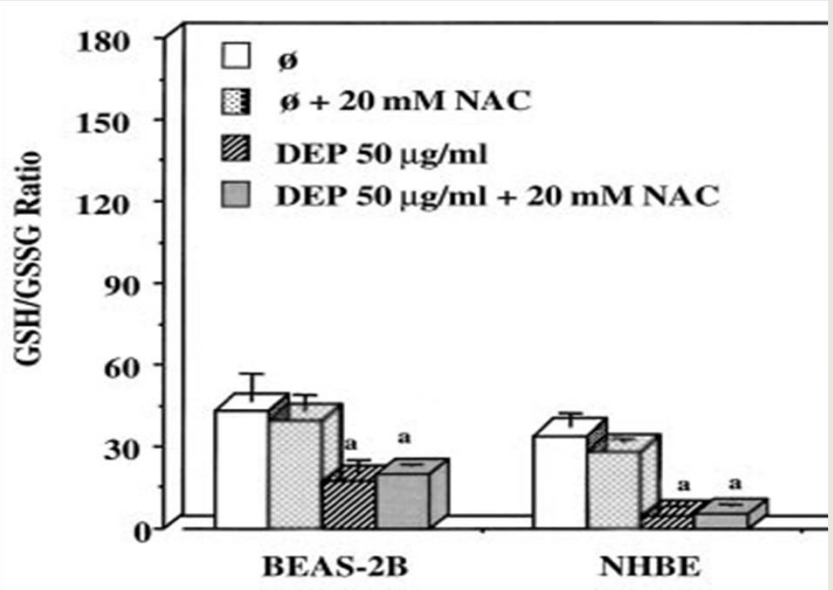
# Assessment of ROS

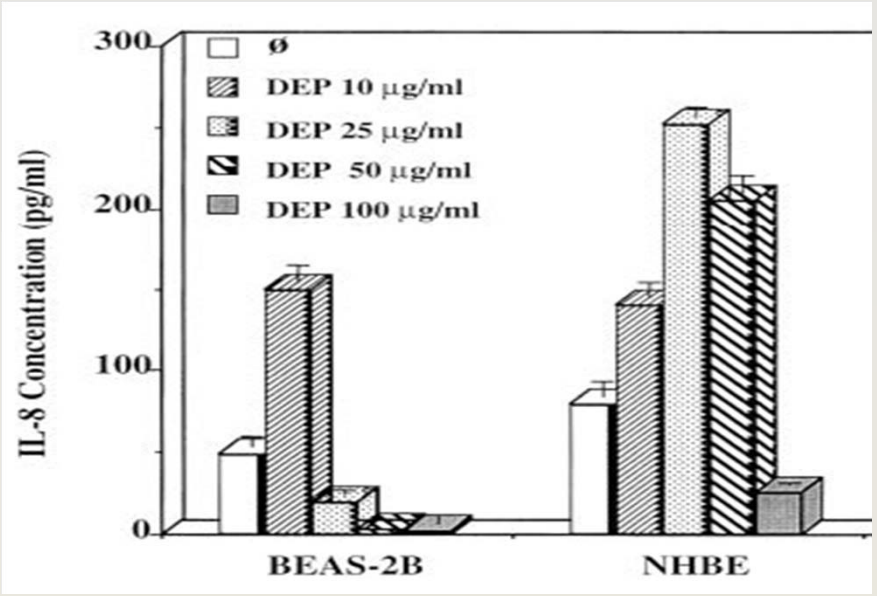
- Direct: $O2^{-}$ , $OH^{-}$
- Indirect: Colorimetric assays (e.g. NBT), use of probes (e.g. DCF, HE)
- Oxidative products
  - Lipids: MDA/TBARS, lipid hydroperoxides, F2-isoprostanes, HETEs, HODEs
  - Proteins: carbonyls
  - DNA: 8-oxodG, 8-oxoGua, M1dG
- Gene expression:
  - Prooxidant genes: NADPH oxidase
  - Antioxidant genes: Nrf2, HO-1, SOD, etc. determined by qPCR, WBs, IH.

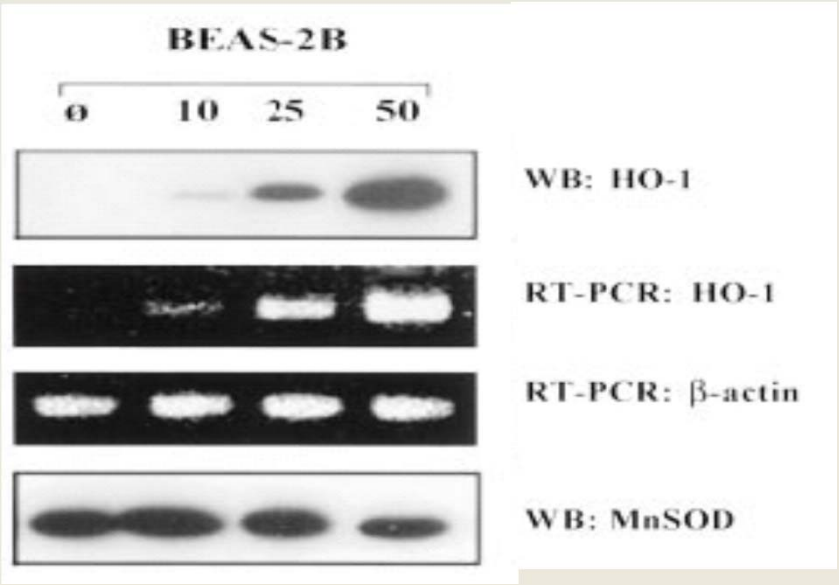
# Assessment of Inflammation

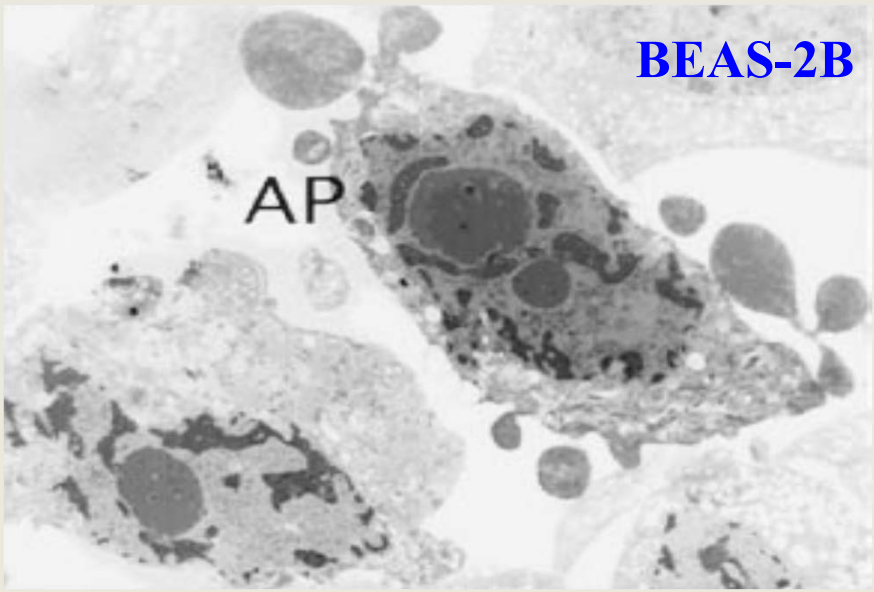
- Histology
  - Lung infiltration by inflammatory cells: degree, type of cells, localization
  - BALF total cell count/cell differential
  - Vascular infiltration of inflammatory cells, atherosclerosis
- Chemistry/Biochemistry
  - Inflammatory mediators: TNF-α, MCP-1, IL-6, IL-8, CAMs (VCAM-1, ICAM-1) …
  - BALF total protein/albumin, LDH
- Gene expression of inflammatory mediators and signaling pathways by qPCR, WBs or IH.

# Epithelial cells



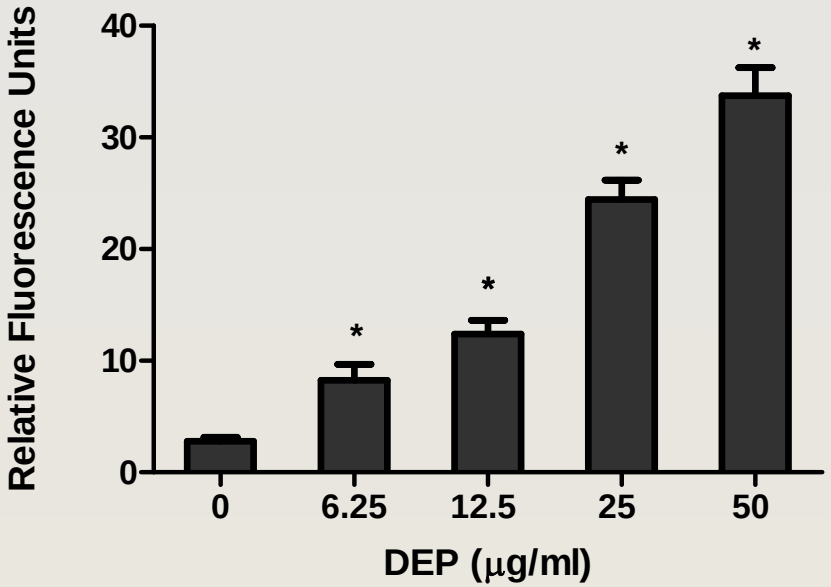



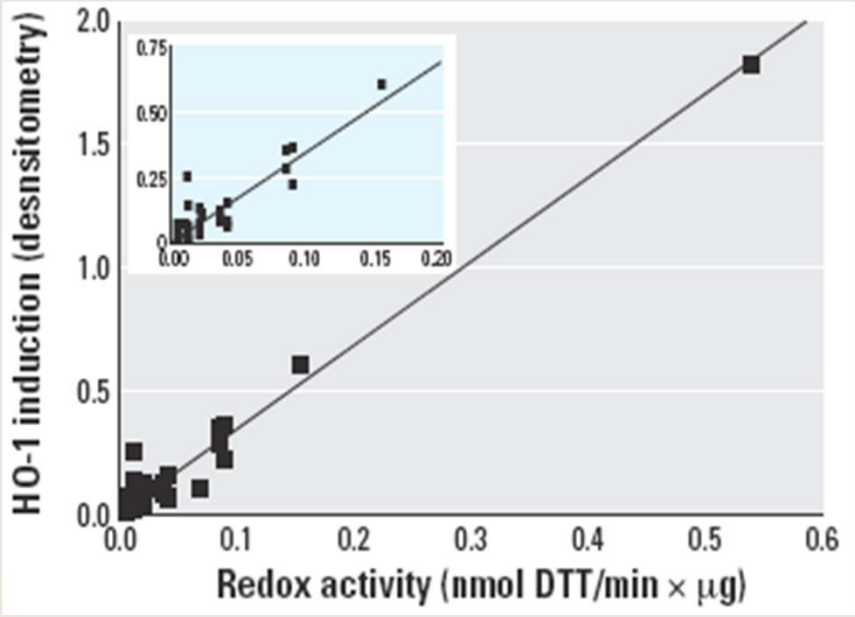



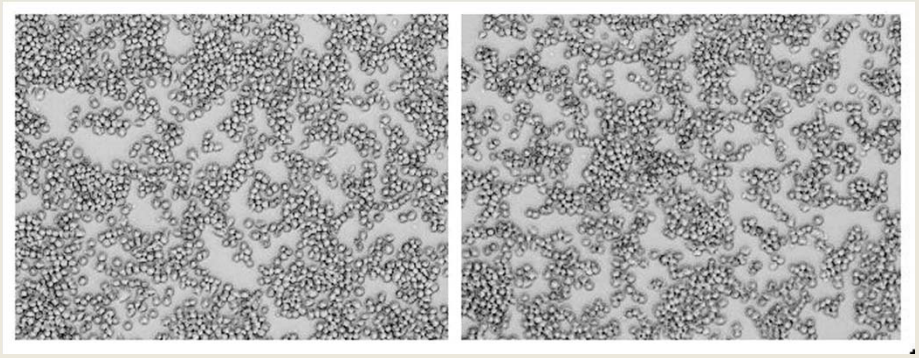

*Li et al, JI 2002; 169: 4531*

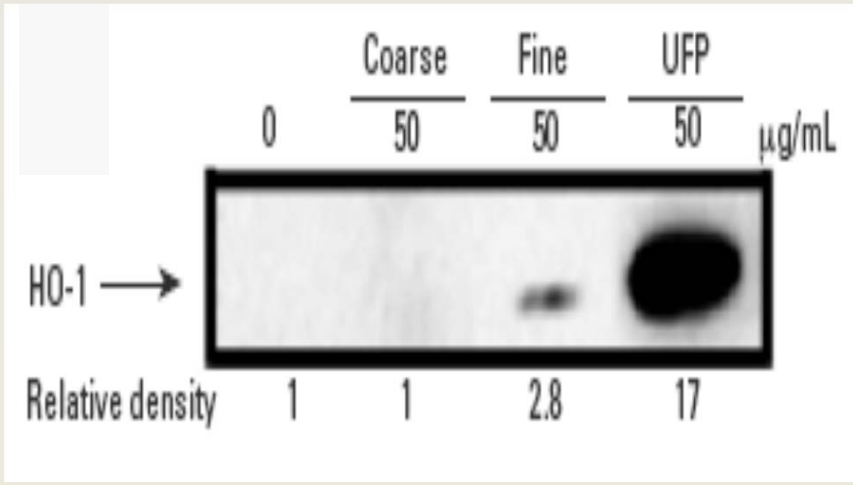
# Macrophages




**DCF only** **DEP 12.5 μg/ml**

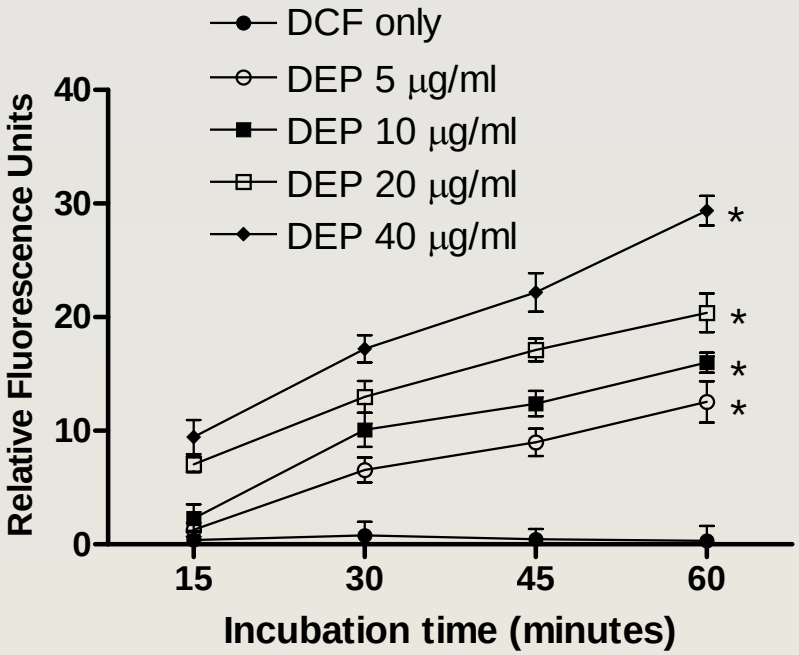


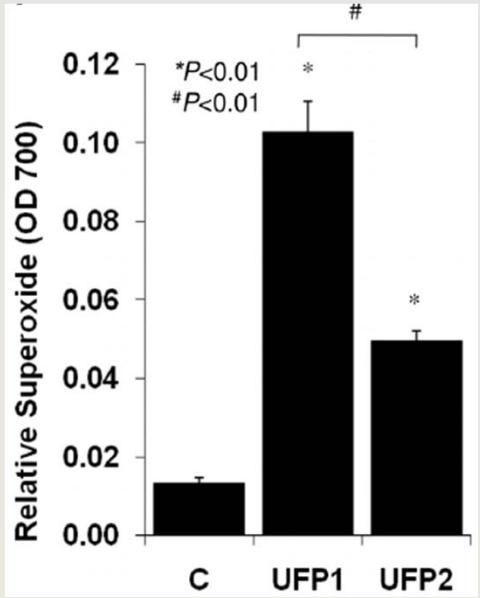


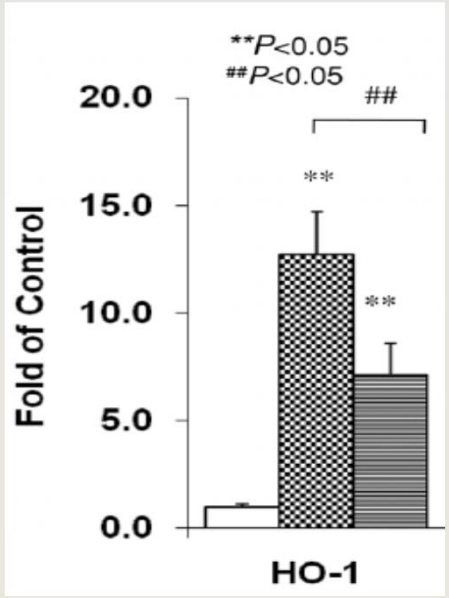

*Yin et al, JBMT 2013; 27: 172*

*Li et al, EHP 2003; 111: 455*

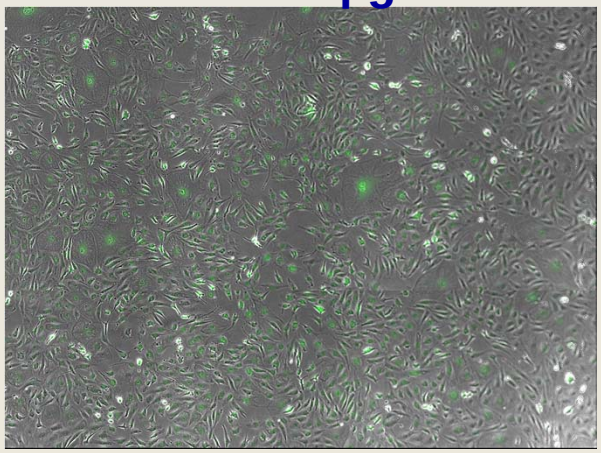
# Endothelial cells

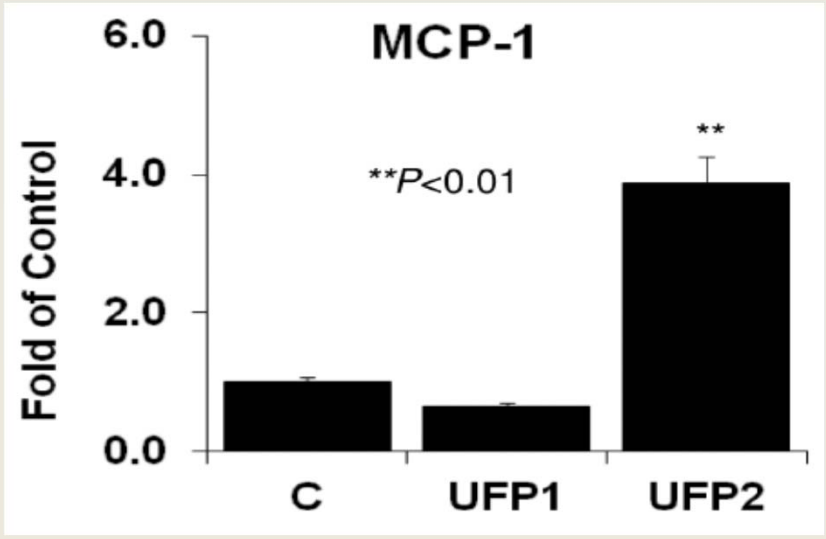



**DEP 10 μg/ml**






*Yin et al, JBMT 2013; 27: 172*

*Li et al, PFT 2010; 7: 6*

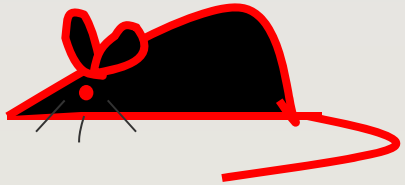
# PM Exposure, ROS and Inflammation In-Vivo

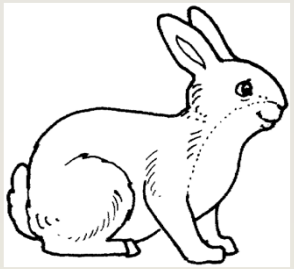
**I.P./I.T. Instillations**

- **PM**
- **DEP**
- **ROFA**
- **Carbon**

**Inhalation Exposures**

- **CAPs**
- **Reaerosolized particles**
- **DE, GE**





## Pulmonary Effects

BALF

Lung Tissue

?

## Systemic Effects

Circulating Blood

Metabolism

Vasculature

Solid Organs:
Liver, Pancreas,
Kidneys

# UFP Induce Pulmonary and Systemic Oxidative Stress


HO-1 Tg Luc mice

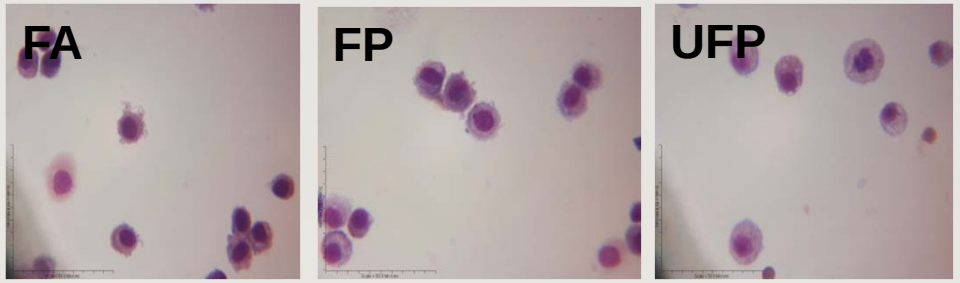
Chow

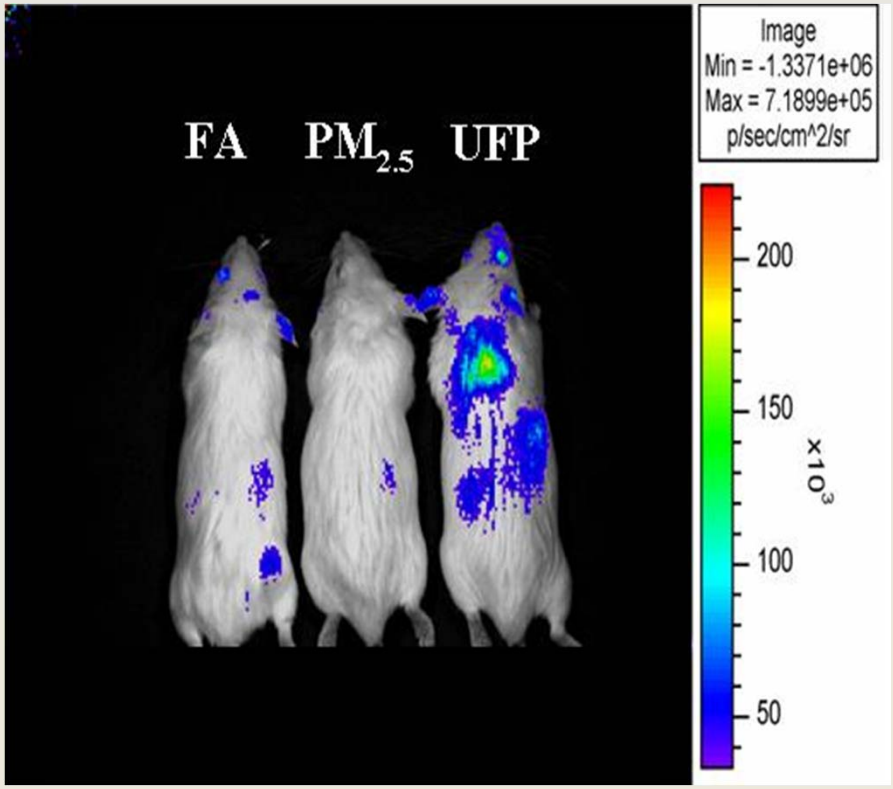
5 hours

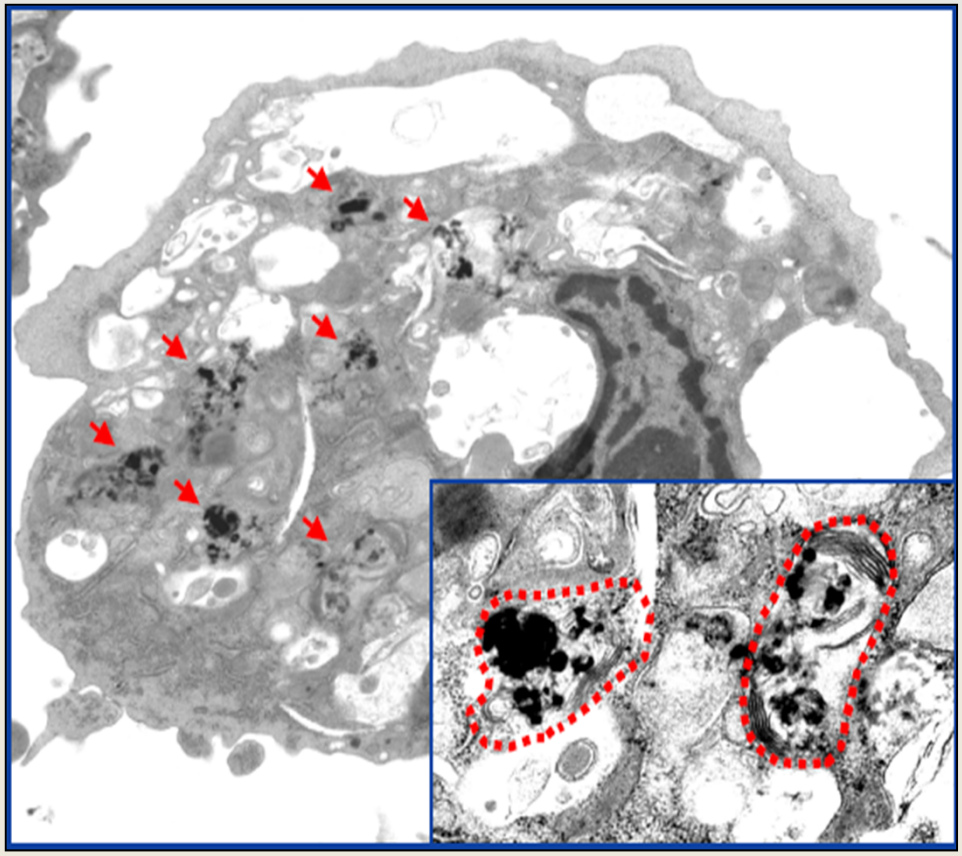
Filtered air (FA)

PM<2.5µm (FP)

PM<0.18µm (UFP)

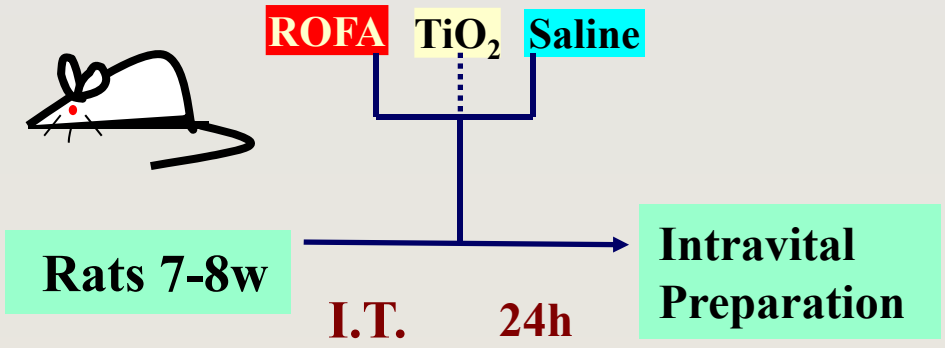


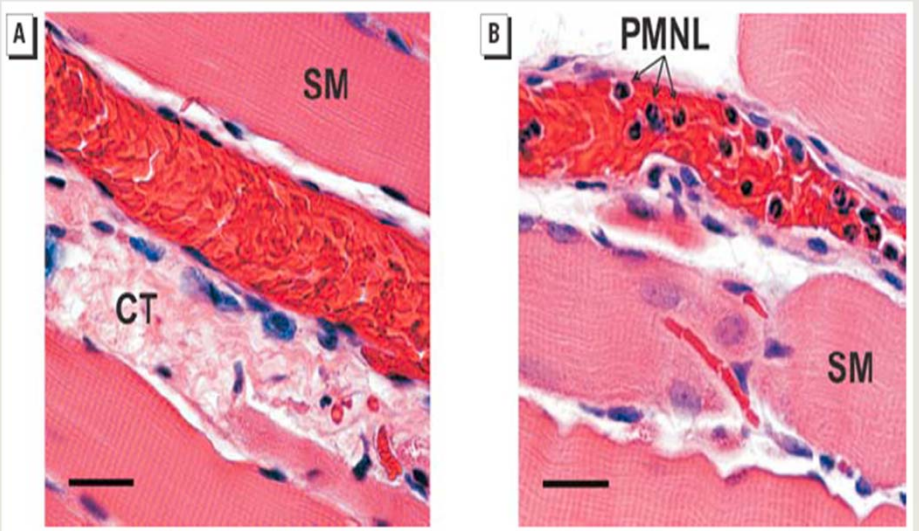



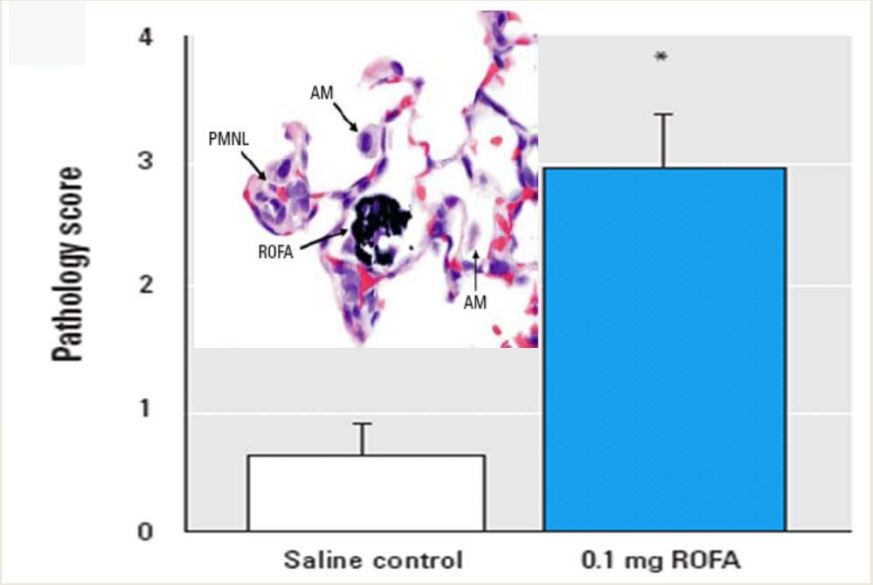



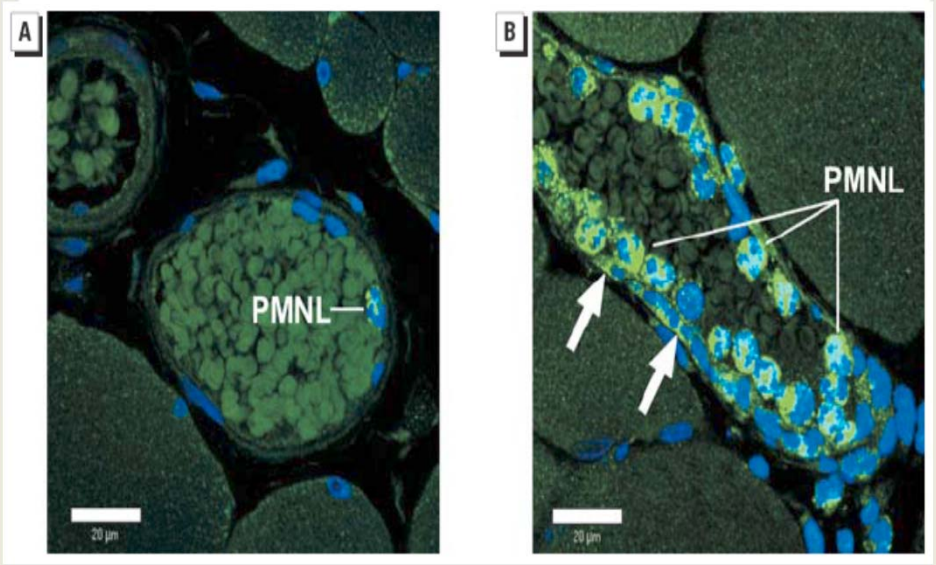
*Araujo et al, Part Fibre Toxicol 2009*

# PM causes endothelial dysfunction and systemic vascular inflammation



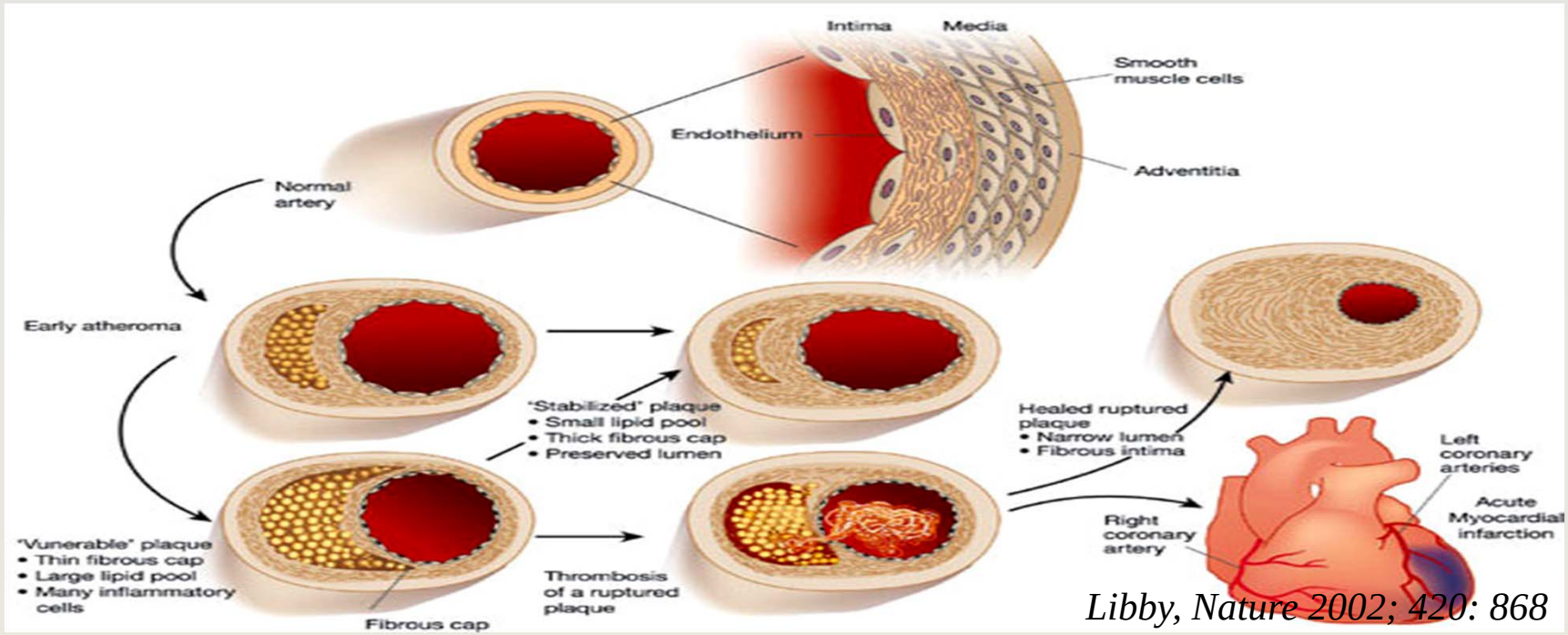



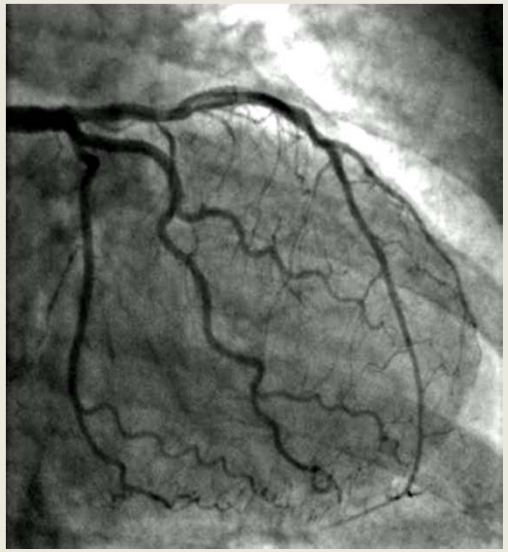



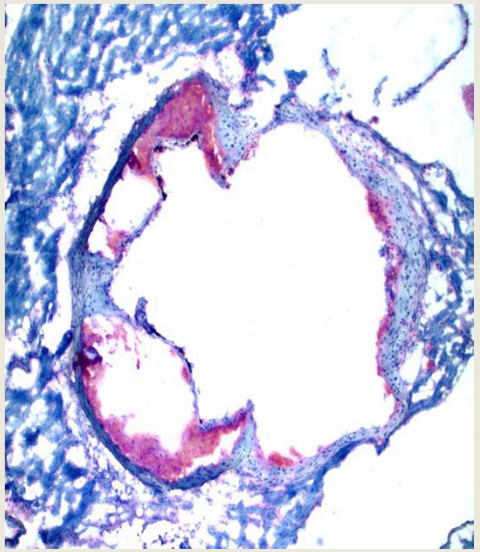

*Nurkiewicz et al, EHP 2006; 114: 412*

# Assessment of Atherosclerosis

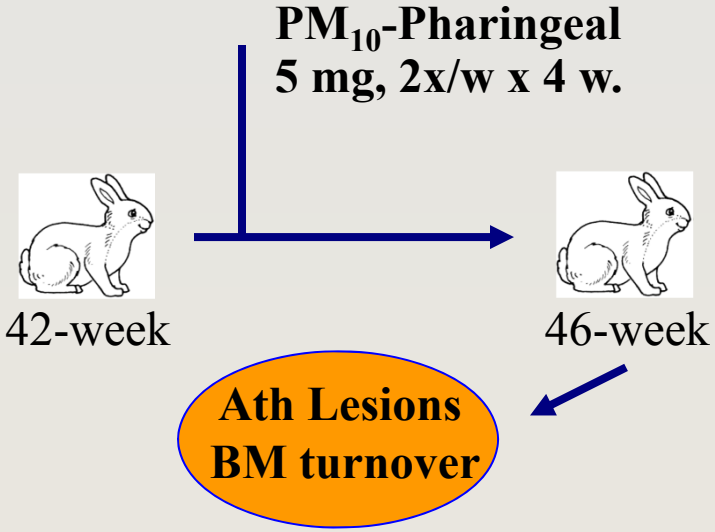


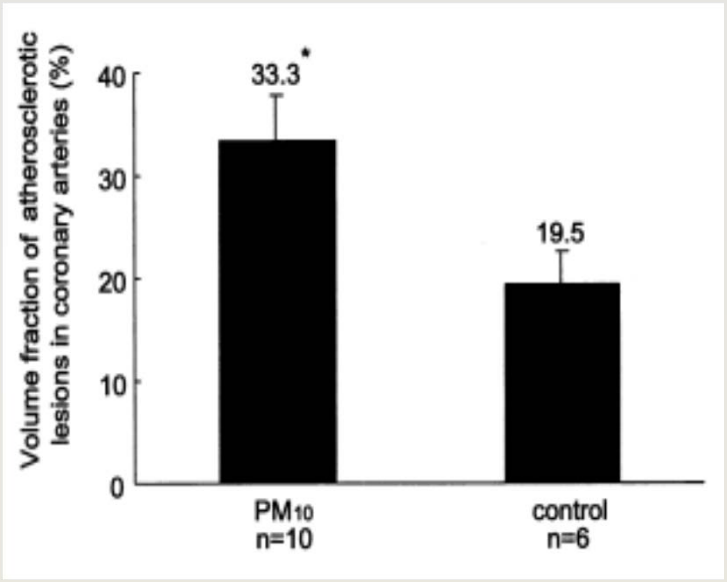

*Libby, Nature 2002; 420: 868*

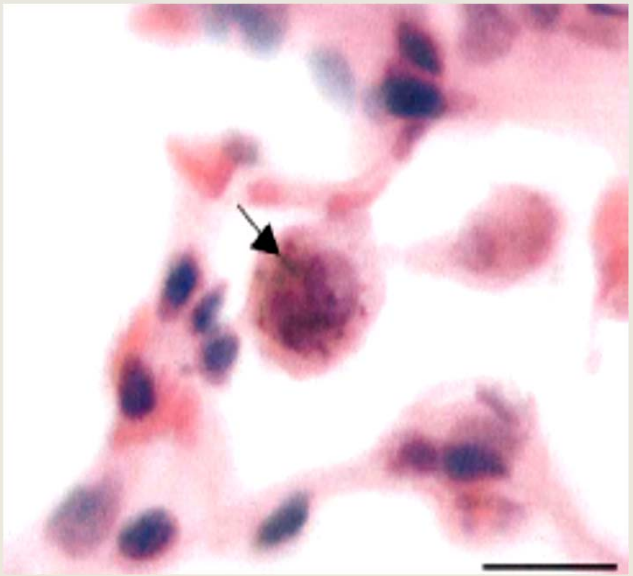


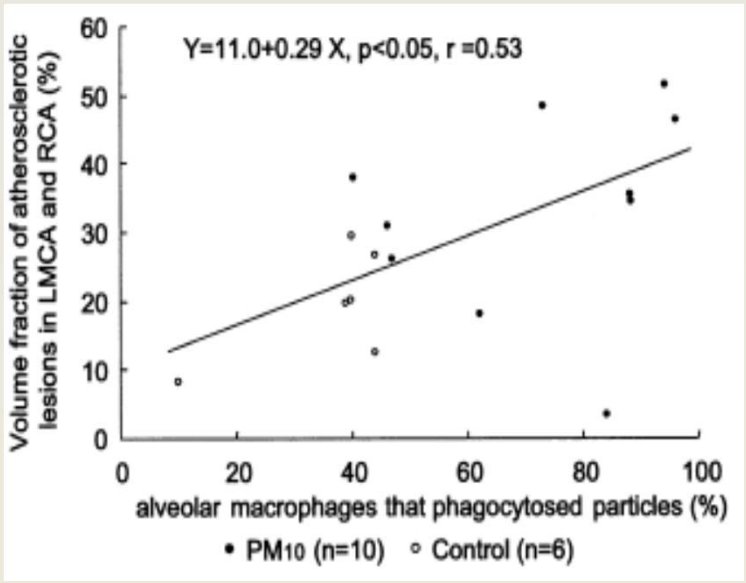


# $PM_{10}$ enhances atherosclerosis in rabbits






*Suwa et al, JACC 2002; 39: 935*

# $PM_{2.5}$ Promotes Atherosclerosis in ApoE KO

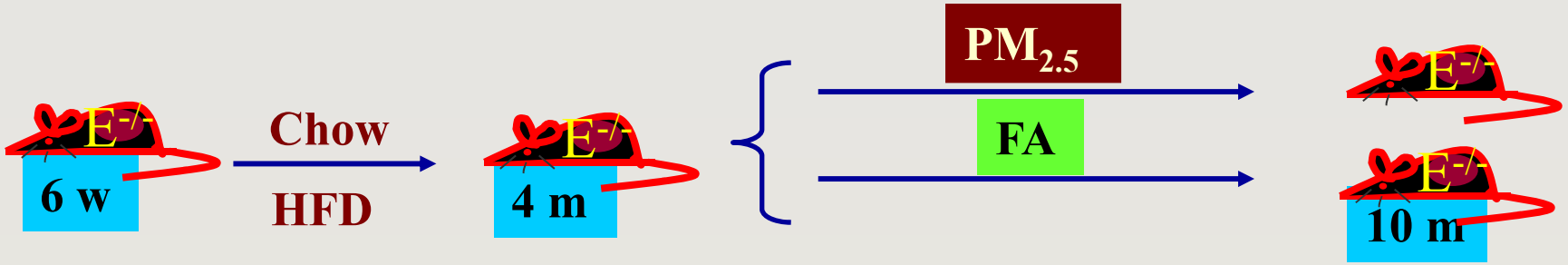
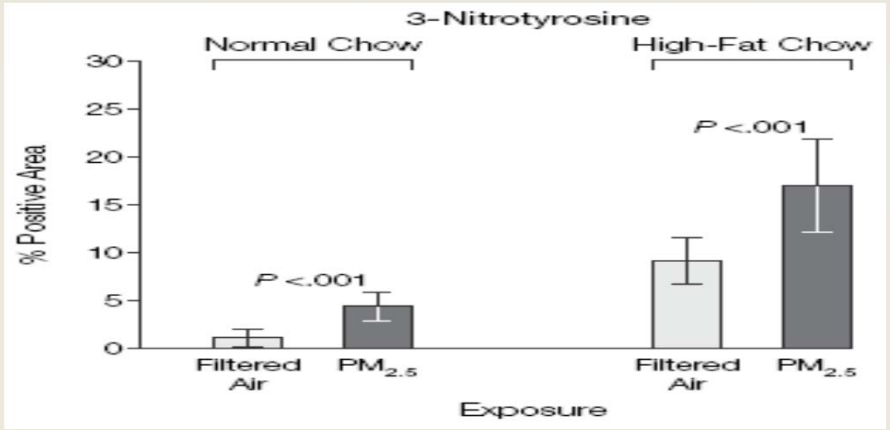



**Table 3.** Analysis of Plaque and Immunohistochemical Staining Parameters*

| | Normal Chow, Mean (SD) | | | High-Fat Chow, Mean (SD) | | |
|---|---|---|---|---|---|---|
| Staining | Filtered Air | $PM_{2.5}$ | *P* Value† | Filtered Air | $PM_{2.5}$ | *P* Value† |
| Plaque area, % | 13.2 (8.1) | 19.2 (13.1) | .15 | 26.2 (8.6) | 41.5 (9.8) | <.001 |
| Oil red-O | 10.0 (4.1) | 15.3 (11.8) | .13 | 20.0 (7.0) | 30.0 (8.2) | .02 |
| CD68 | 7.0 (2.2) | 12.8 (3.7) | <.001 | 13.0 (1.4) | 19.5 (4.5) | <.001 |
| 3-Nitrotyrosine | 1.1 (0.8) | 4.4 (1.5) | <.001 | 9.0 (2.5) | 16.9 (4.9) | <.001 |
| Endothelial NOS | 0.6 (0.3) | 1.1 (0.5) | .06 | 3.5 (0.7) | 4.7 (1.1) | .07 |
| Inducible NOS | 0.8 (0.5) | 3.2 (0.9) | <.001 | 4.9 (1.1) | 13.0 (3.6) | <.001 |

## Aortic Oxidative Stress




*Sun et al, JAMA 2005; 294: 3003*

# UFP Enhance Atherosclerosis in ApoE null mice


6-week-old male

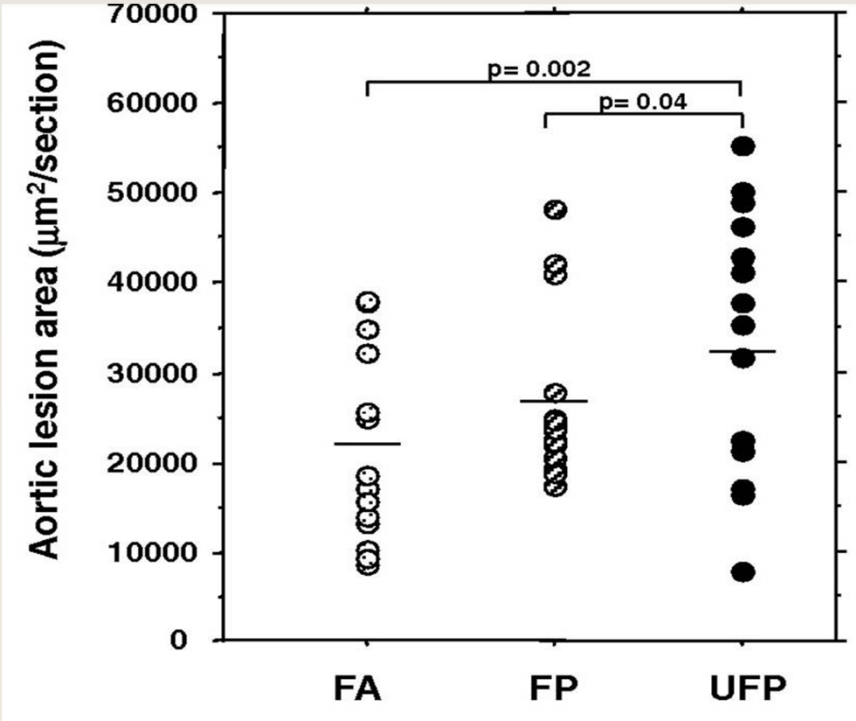
Chow

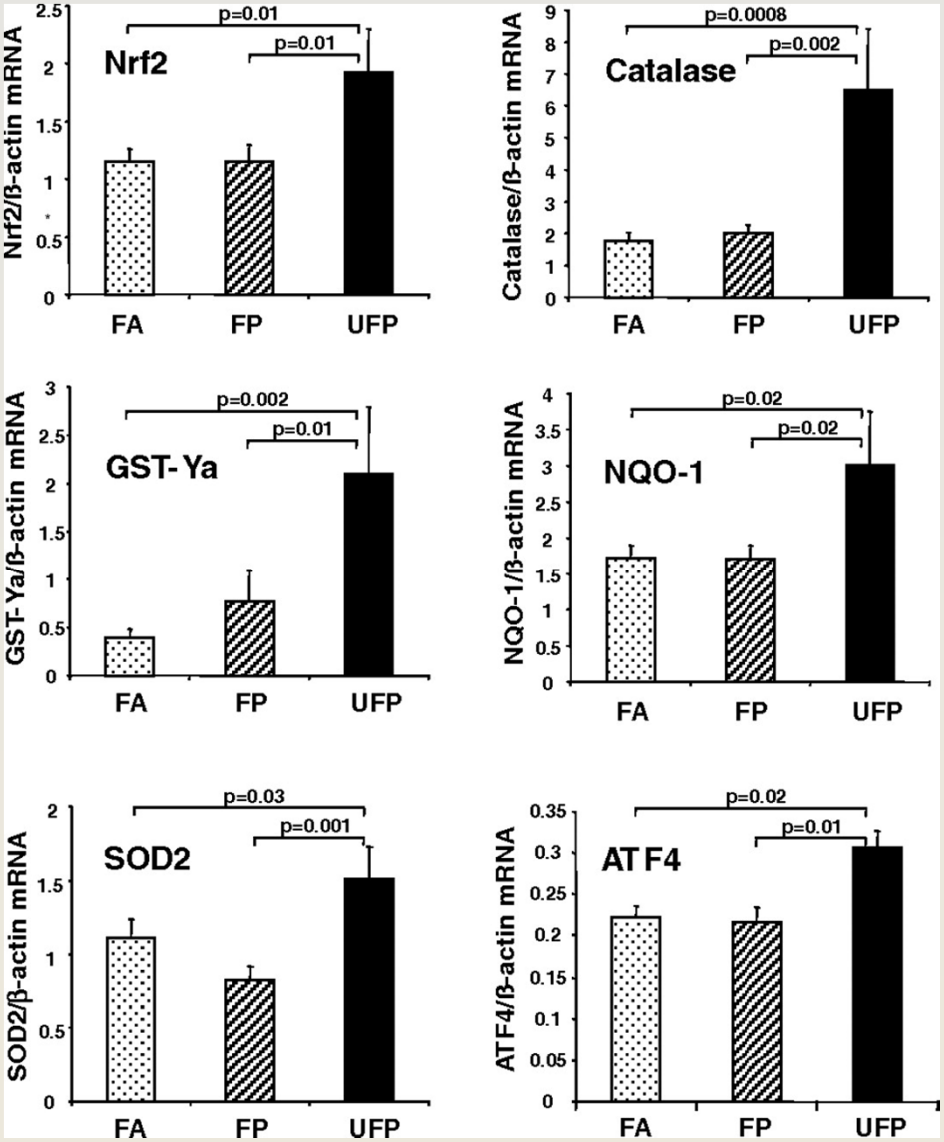
Exposures: 5 hours/session
3x/week for 5 weeks

Filtered air (FA)

PM<2.5µm (FP)

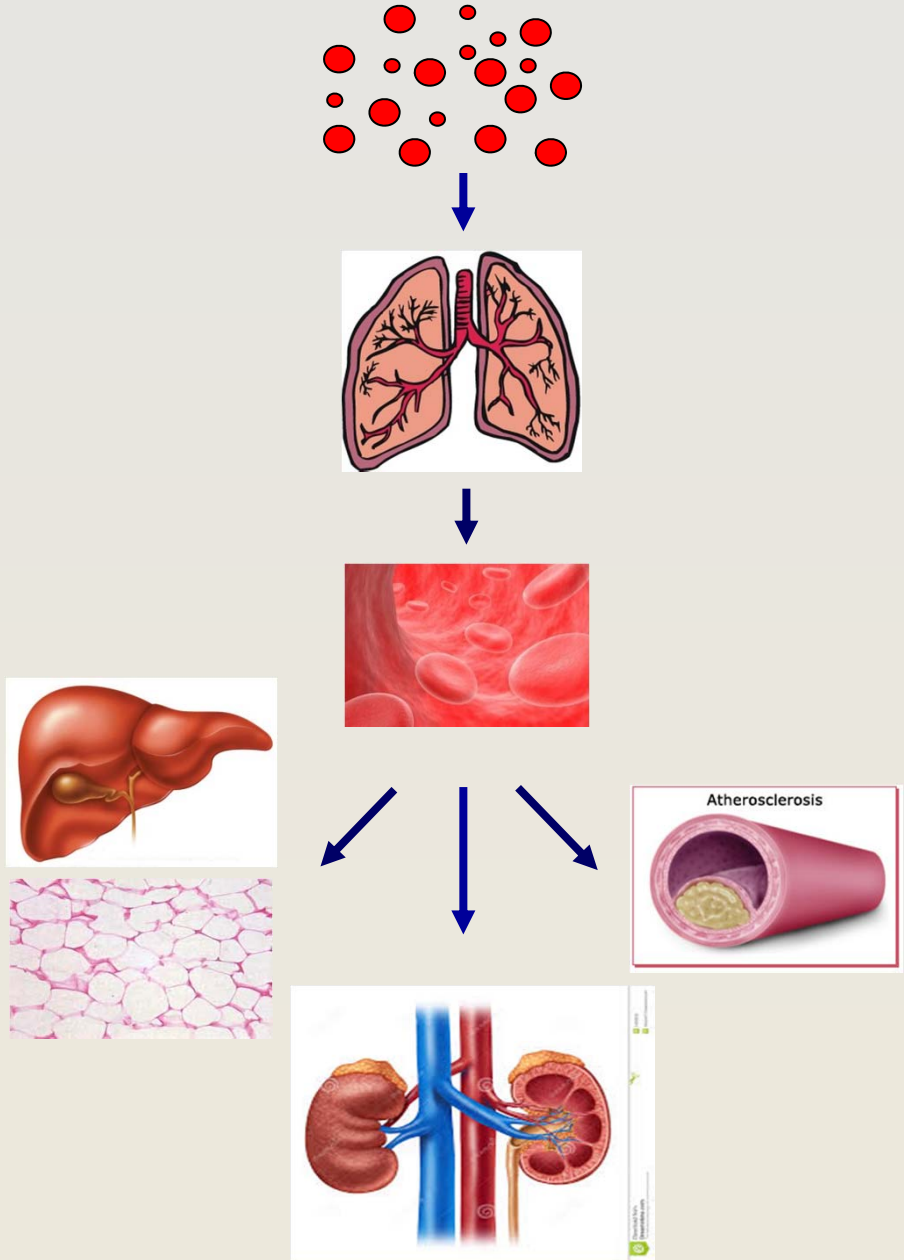
PM<0.18µm (UFP)



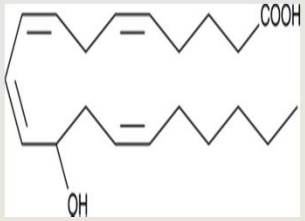




*Araujo et al, Circ Res 2008; 102: 589*

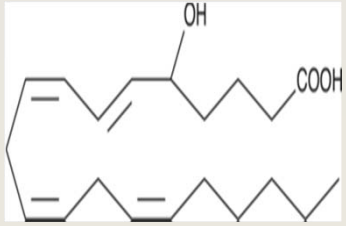
# New Investigational Approaches



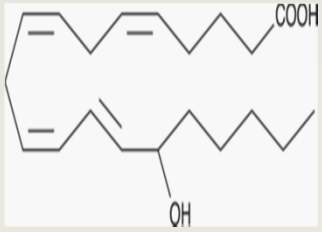
# Lipid Peroxidation

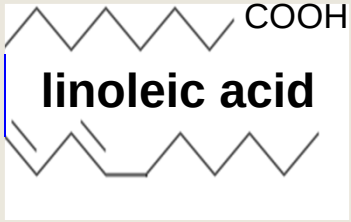

12-HETE




5-HETE

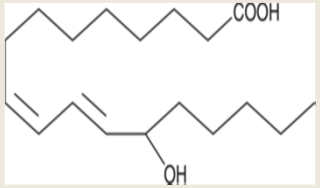



15-HETE

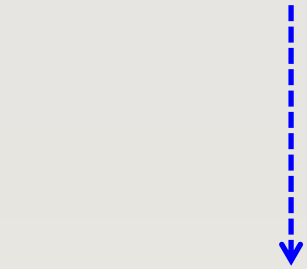


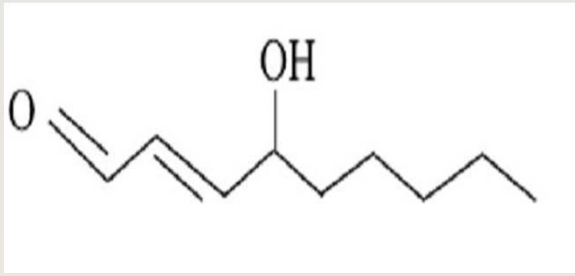


9-HODE

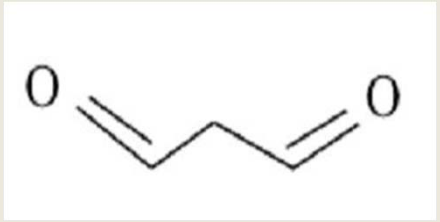



13-HODE



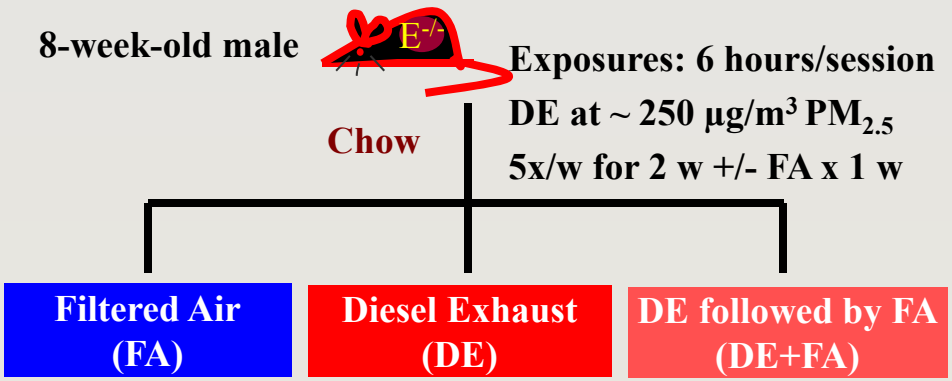


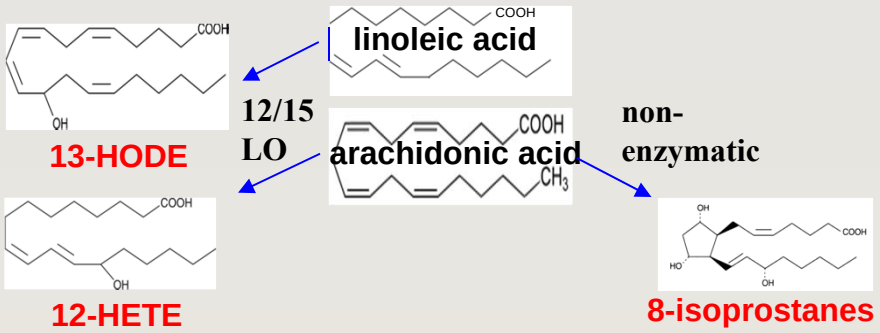

4-HNE

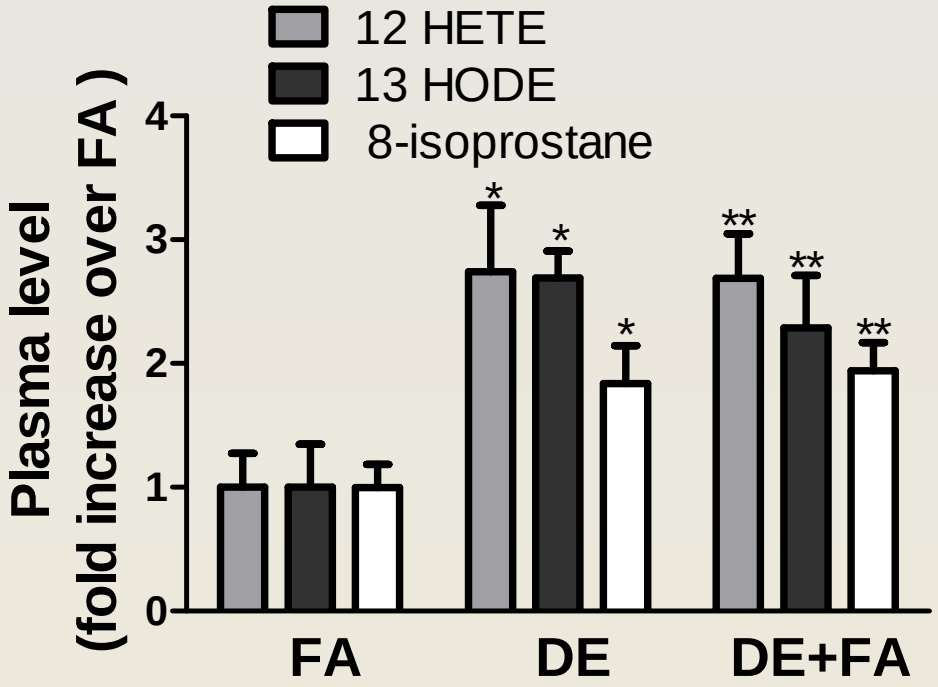



MDA

# PM Enhances Lipid Peroxidation in the Blood



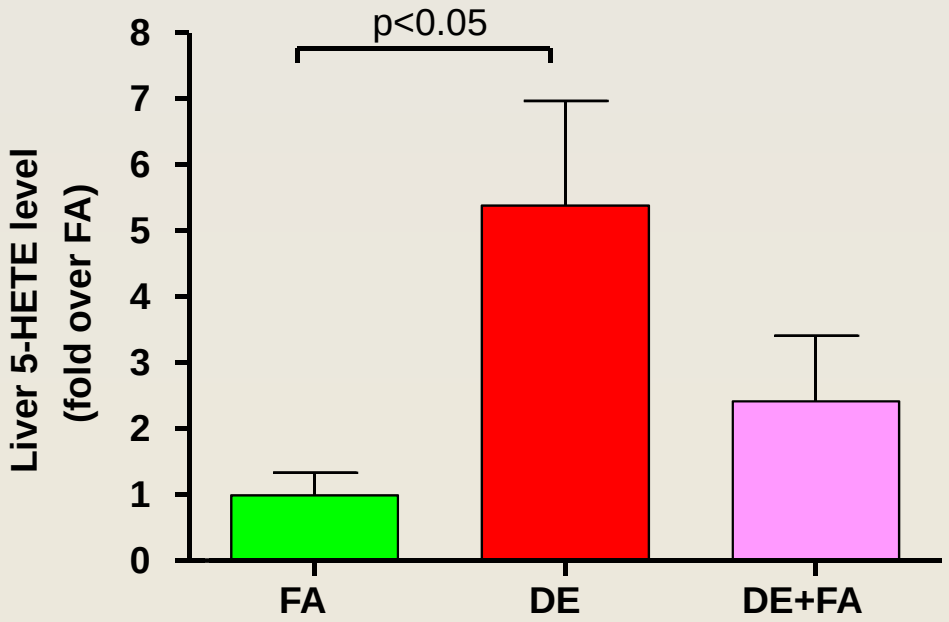




*Yin et al, ATVB 2013; 33: 1153*

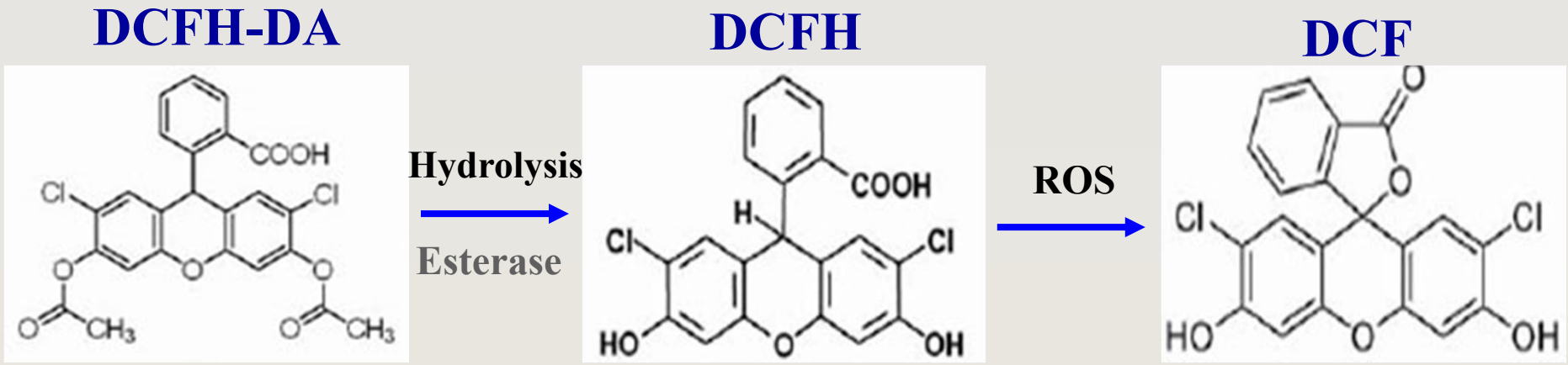
# PM Promotes Lipid Peroxidation in the Liver

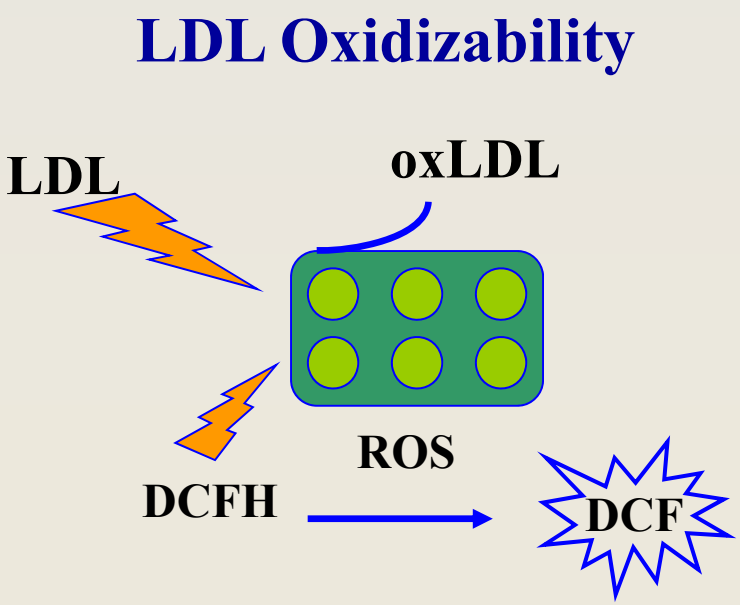
## Hepatic levels of HETEs and HODEs

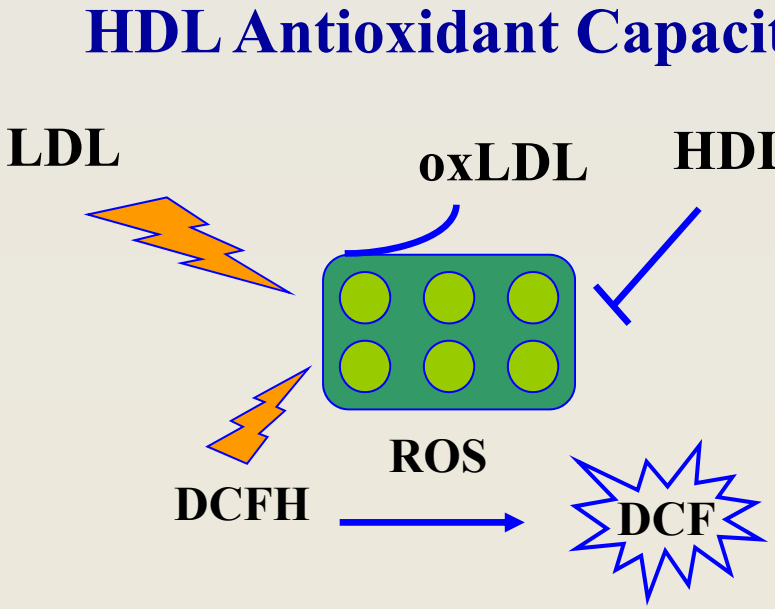
| Liver (ng/ml) | FA | DE | DE+FA |
|---|---|---|---|
| 5-HETE | 10.6±3.70 | 56.9±17.0 * | 25.3±10.1 |
| 12-HETE | 111.1±38.0 | 119.1±14.0 | 102.9±13.4 |
| 15-HETE | 84.1±8.13 | 93.7±14.7 | 108.4±7.05 |
| 9-HODE | 1413.7.5±130.1 | 1304.2±150.3 | 1365.3±49.8 |
| 13-HODE | 1247.5±192.7 | 989.1±188.7 | 1357.8±105.8 |



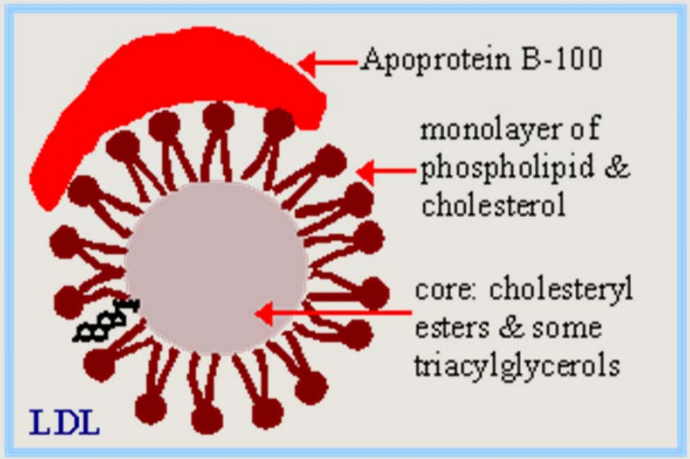
# Assessment of Plasma Lipoproteins

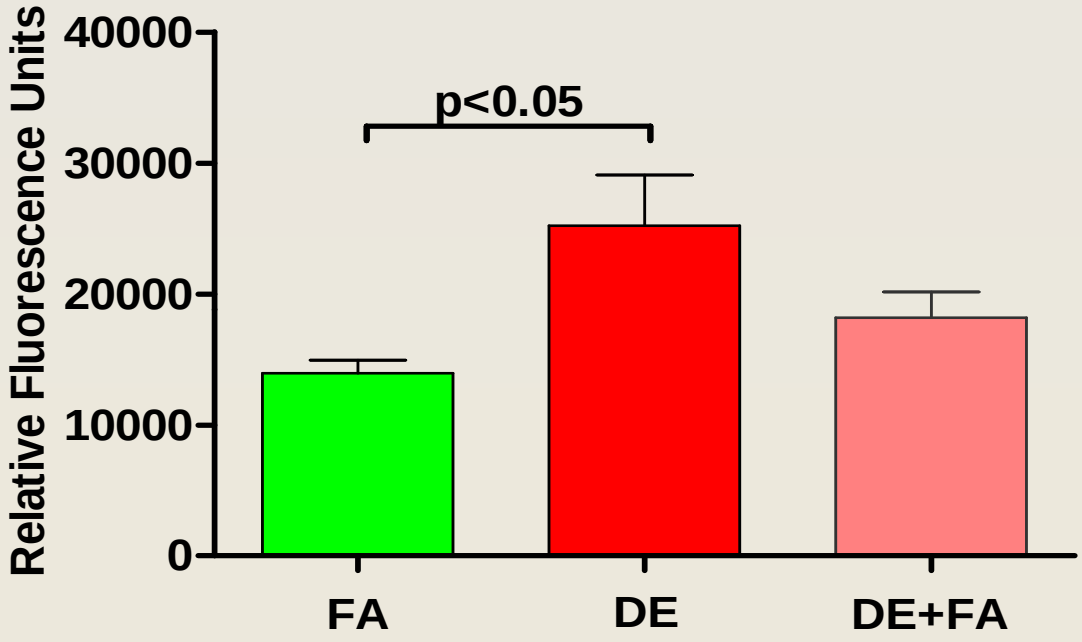


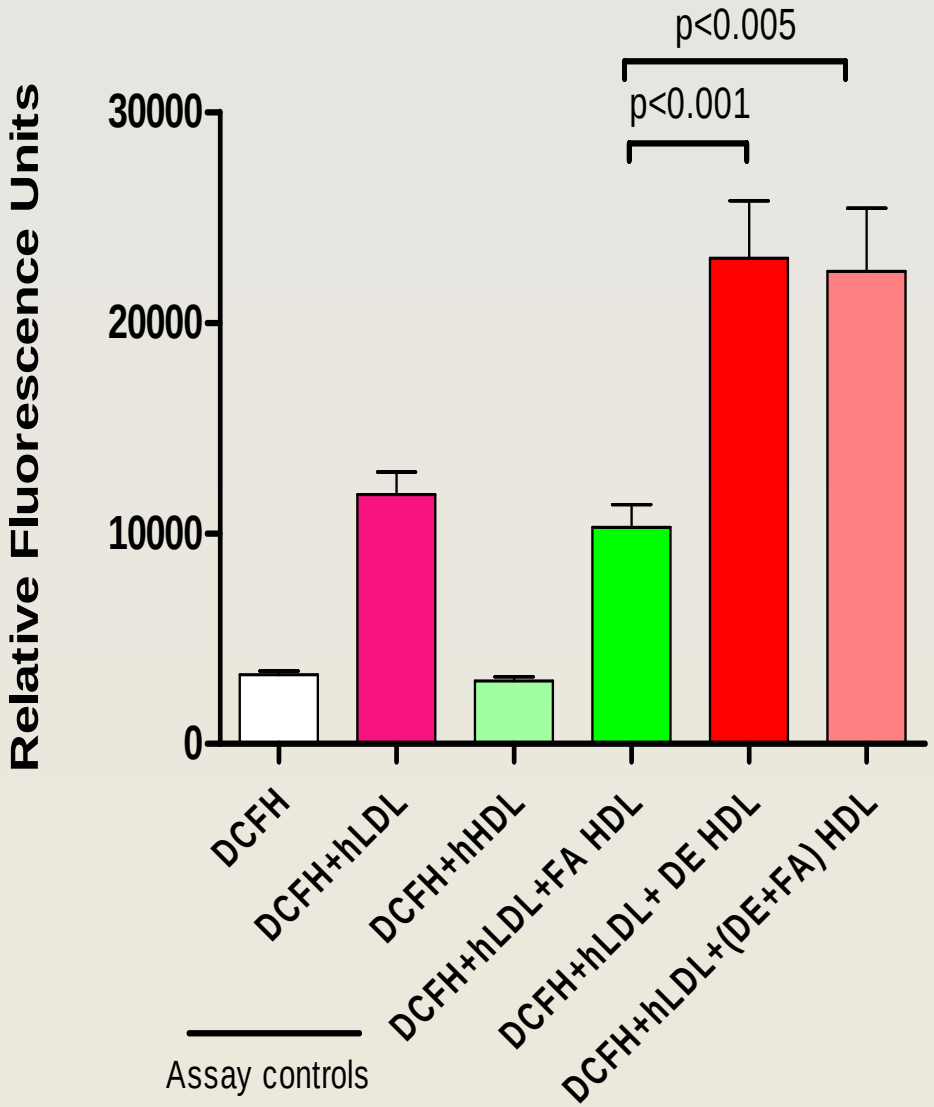



# PM Increases the Oxidizability of VLDL+LDL Lipoproteins

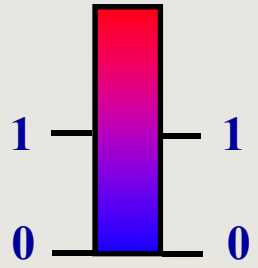


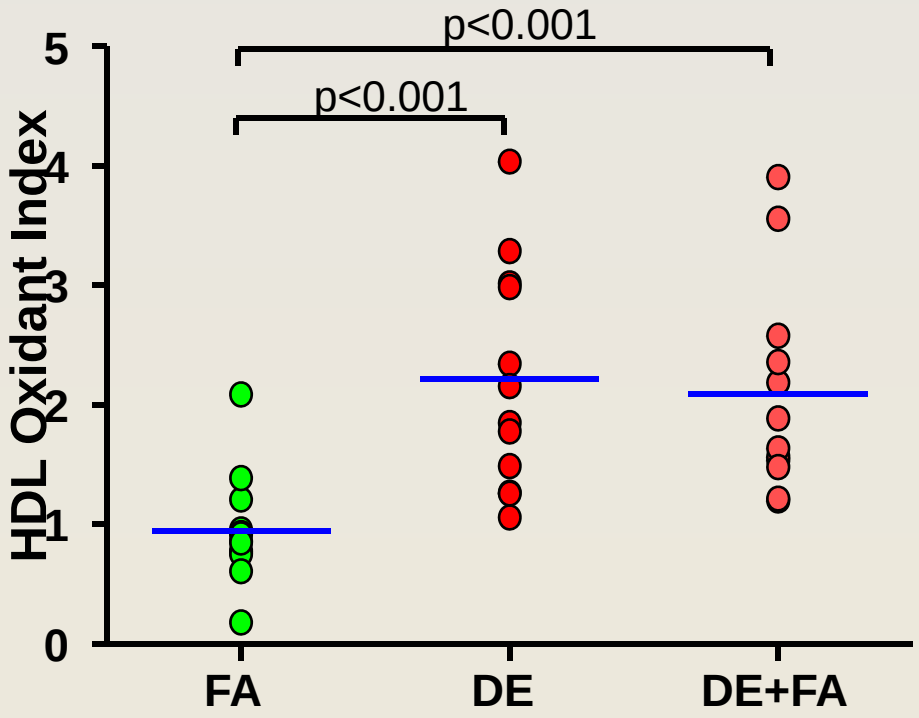



# PM Exposures lead to Prooxidative HDL




$$HOI = \frac{FU\ (+\ HDL)}{FU\ (-\ HDL)}$$




*Yin et al, ATVB 2013; 33: 1153*

# Outline

I) Air Pollution and Health

II) Toxicological Evidence

a. Approaches to study health effects

b. Effects in the lungs, vasculature, metabolism

c. New investigational approaches

**III) Summary and Perspectives**

# Summary

- ✓ Inhalation of air pollutants induce a variety of health effects resulting in increased morbidity and mortality.
- ✓ Health effects are diverse and affect many organs and systems.
- ✓ Both gaseous and particulate constituents are toxic.
- ✓ Most of the mortality is due to cardiovascular and cerebrovascular diseases.
- ✓ There is need to discover/develop novel biomarkers of exposures and biomarkers of health effects.

# Effects of Common Air Pollutants

## RESPIRATORY EFFECTS

**Symptoms:**
- Cough
- Phlegm
- Chest tightness
- Wheezing
- Shortness of breath

**Increased sickness and premature death from:**
- Asthma
- Bronchitis (acute or chronic)
- Emphysema
- Pneumonia

**Development of new disease**
- Chronic bronchitis
- Premature aging of the lungs

## CARDIOVASCULAR EFFECTS

**Symptoms:**
- Chest tightness
- Chest pain (angina)
- Palpitations
- Shortness of breath
- Unusual fatigue

**Increased sickness and premature death from:**
- Coronary artery disease
- Abnormal heart rhythms
- Congestive heart failure


## How Pollutants Cause Symptoms

**Effects on Lung Function**
- Narrowing of airways (bronchoconstriction)
- Decreased air flow

**Airway Inflammation**
- Influx of white blood cells
- Abnormal mucus production
- Fluid accumulation and swelling (edema)
- Death and shedding of cells that line airways


**Increased Susceptibility to Respiratory Infection**

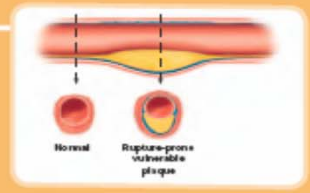
Normal

Lung with respiratory infection

## How Pollutants May Cause Symptoms


**Effects on Cardiovascular Function**
- Low oxygenation of red blood cells
- Abnormal heart rhythms
- Altered autonomic nervous system control of the heart

**Vascular Inflammation**
- Increased risk of blood clot formation
- Narrowing of vessels (vasoconstriction)
- Increased risk of atherosclerotic plaque rupture

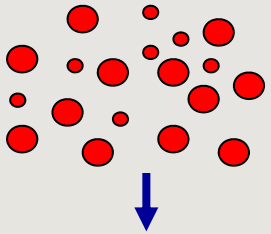


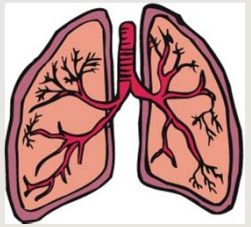

**Reduce your risk by using the Air Quality Index (AQI) to plan outdoor activities – www.airnow.gov**

| AQI Levels of Health Concern | AQI Values | What Action Should People Take? |
|---|---|---|
| Good | 0-50 | Enjoy Activities |
| Moderate | 51-100 | People unusually sensitive to air pollution:<br>Plan strenuous outside activities when air quality is better |
| Unhealthy for Sensitive Groups | 101-150 | Sensitive Groups: Cut back or reschedule strenuous outside activities<br>Particle Pollution: People with heart or lung disease (including diabetics), older adults, and children<br>Ozone: Active children and adults and people with lung disease<br>Sulfur Dioxide: Active children and adults with asthma<br>Carbon Monoxide: People with heart disease and possibly fetuses and infants |
| Unhealthy | 151-200 | Everyone: Cut back or reschedule strenuous outside activities<br>Sensitive groups: Avoid strenuous outside activities |
| Very Unhealthy | 201-300 | Everyone: Significantly cut back on outside physical activities<br>Sensitive groups: Avoid all outside physical activities |

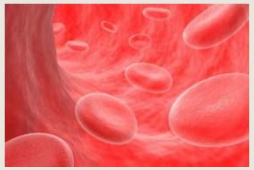
EPA

# Lipid Peroxidation and Biomarkers



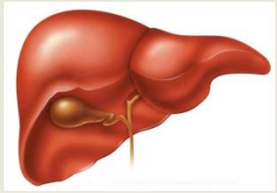


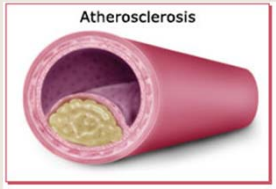
↑ Lipid Peroxidation



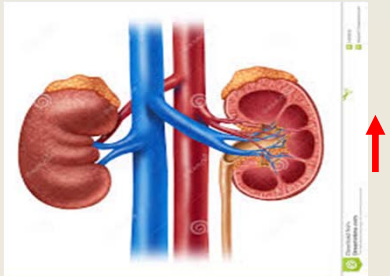
↑ Lipid Peroxidation
Dysfunctional HDL
oxLDL

↑ Lipid Peroxidation
Activation 5-LO



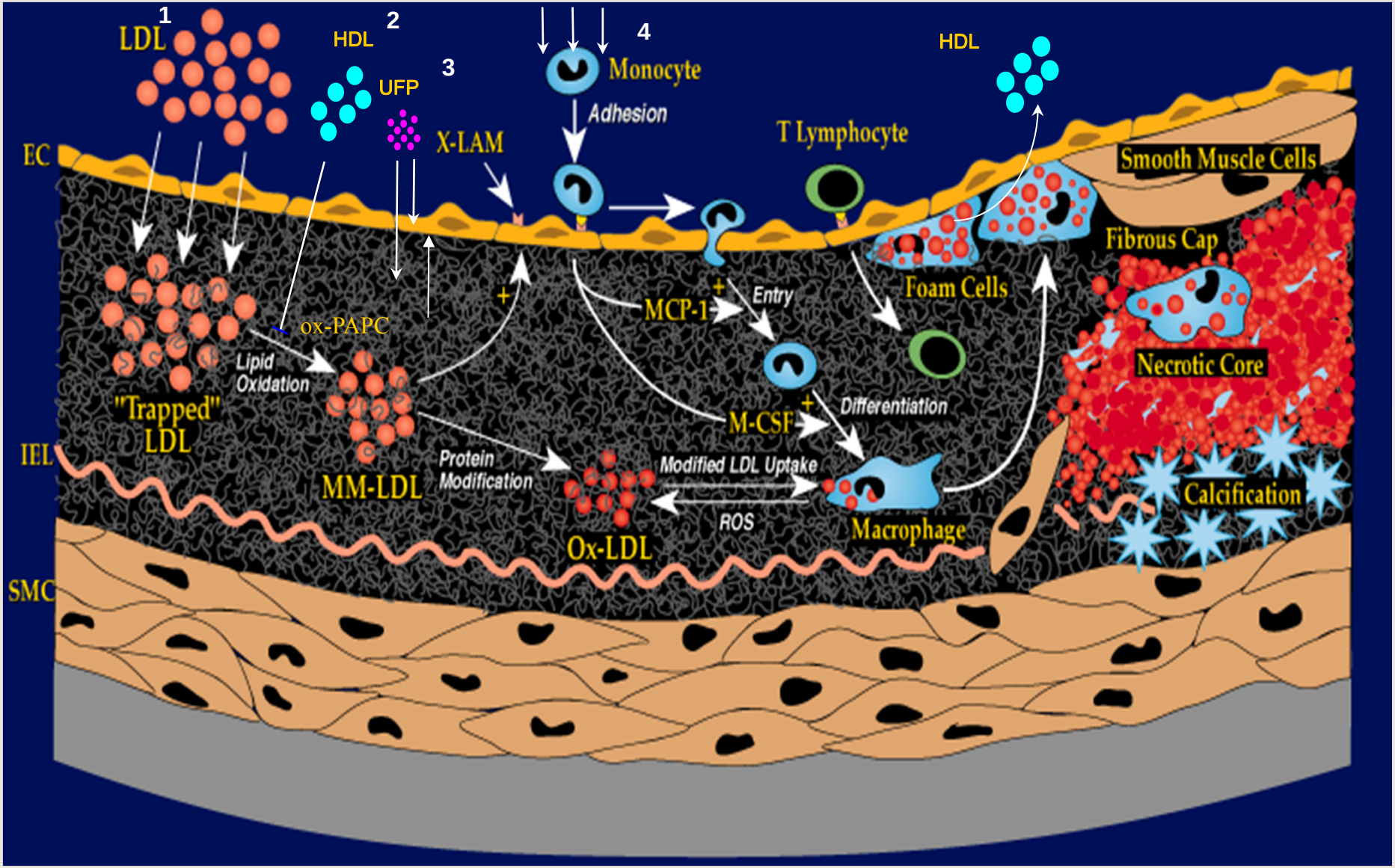



↑ Lipid Peroxidation
↑ Atherosclerosis



↑ Lipid Peroxidation

# Air Pollution and Atherosclerosis




*Araujo & Rosenfeld, In Air Pollution and Health Effects 2015*

# Perspectives